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THE BACTERIAL PRECIPITINS.*

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INTRODUCTORY.

A LONG series of complicated studies upon the body fluids made during the past few years has led to the discovery of a number of hitherto unknown or little understood substances which seem to be of great significance in the processes of immunization of man and the lower animals to bacteria or to their toxins, and in the adaptation of the body to a great variety of alien materials. Many of these substances are present in the blood serum under normal conditions, and they may be increased in amount, or entirely new substances, it seems, may be formed when the body adapts itself to foreign material artificially introduced into the veins, subcutaneous tissue, or the peritoneal cavity, or after adap-

tation following upon the course of a bacterial infection or disease. The substances, whether normally present in serum or developed as a result of adaptation, are commonly designated "antibodies," and the serum containing them is known as an "antiserum." For example, the serum of a normal rabbit may contain a substance which agglutinates the typhoid bacillus—an "agglutinin" specific within certain limits for this bacillus. The normal agglutinating substances are, however, greatly increased in amount or in their activity by the adaptation of the rabbit to the typhoid bacillus or to its metabolic products artificially introduced into the body of the animal; or, if the agglutinin be not normally present, it may be formed *de novo* in response to the process of artificial adaptation. The agglutinin is an example of an antibody, and the serum in which it is developed is called an antityphoid serum.

Besides bacteria and their products, various animal or vegetable substances, generally albuminous in nature, such as alien blood sera, serous fluids, or their isolated albumins and globulins, as well as milk, peptone (?), egg albumin, various ferments, and the cells of various organs, or their extracts, derived from different animals, induce, after introduction into the animal organism, the formation of antibodies. These are of several kinds; thus, specific antitoxic, bactericidal, cellucidal, agglutinating, and precipitating properties have been demonstrated in sera.

As a result of the adaptation of animals to serum containing these antibodies, a second series of substances may in many instances be developed which neutralize or render inactive these antibodies. In this manner, antiagglutinins, antiprecipitins, anticoagulins, antiamboceptors (antilynsins), etc., are said to be formed.

Rudolf Kraus,¹ April 30, 1897, described a class of hitherto undiscovered substances in the sera of animals immunized or adapted to certain species of bacteria. Kraus found that cholera, plague, and typhoid antisera, when added to their corresponding bacterial filtrates, cause precipitates. The substance of the serum which causes the precipitation was called a precipitin. On adding an antiserum, say of typhoid, to the heterologous filtrates of plague or cholera, for example, no such reaction, however, took place. The reaction between these antisera and their correspond-

ing filtrates is thus, according to him, strictly specific, and hence as diagnostic as the Gruber-Durham reaction of agglutination was at that time considered to be. This view for the bacterial precipitins has never been seriously questioned,* although A. Wassermann² observes that, as in the case of the agglutinins, bacteria belonging to species closely related possess similar precipitin receptors, indicating that agglutination and precipitation are not strictly specific phenomena. Recently Tupnix,³ in an abstract, has indicated that bacterial precipitins are by no means as specific as one might infer from the articles which had up till then appeared. A few other references of similar import are found in the literature, but they need not be cited.

On the other hand, the researches on non-bacterial precipitins have clearly shown that the precipitins which are developed in an animal of one species after adaptation to the serum of another species are not specific. These hemo- or sero-precipitins, as they are called, thus precipitate, not only the serum of the species of animal by the use of which they have been developed, but also the sera of species more or less closely related. They are, however, markedly special, for a precipitin invariably gives a quicker and more copious reaction in the homologous than in a heterologous serum.

The general analogy existing between the action of the bacterial precipitins and sero-precipitins led the writer to believe that a more careful study than had hitherto been attempted might reveal relationships between the bacterial precipitins similar to those existing between the sero-precipitins, as mentioned above.

Furthermore, the constant association of the bacterial agglutinins and precipitins in antisera, and the striking points of similarity in the action of these two antibodies, seemed to indicate that between the bacterial precipitins relationships would be demonstrated similar to those which had been conclusively shown to exist between the bacterial agglutinins at the time these studies were begun—in the fall of 1901.

Before undertaking the main part of our task upon the inter-

*The term "bacterial precipitin" is used by the writer to indicate the substance in the serum of normal or adapted animals which induces the precipitation of bacterial broths, filtrates, or other culture extracts.

relations of the bacterial precipitins, a series of observations were made to determine the precipitating action of normal rabbit serum upon various bacterial filtrates.

Although bacterial precipitins have not hitherto been demonstrated in normal sera, the fact that they have been found in association with the agglutinins in the serum of animals adapted to different bacterial species—so constantly indeed that some observers consider these antibodies identical substances—seems to indicate that their presence in normal sera may either have escaped attention, or that, if present, they are in quantities so small that their detection is not possible.*

On the other hand, the possibility of precipitates forming in mixtures of such complex fluids as nutrient broth and serum, by some action other than that induced by normal precipitins, cannot be summarily dismissed.

These reasons led the writer to investigate the precipitating action of normal and various antibacterial sera upon plain nutrient broth and upon various bacterial filtrates.† The tests which are now to be described are preliminary, and serve as controls for the subsequent series described later on.

* The precipitins, however, unlike the agglutinins, are infrequently found in appreciable quantities in normal serum. According to Linossier and Lemoine,⁴ the hemo- or sero-precipitins of one species for the serum of other species of warm-blooded animals do not exist in the serum of warm-blooded animals. Noguchi⁵ has, however, found normal precipitins in horse serum, for the serum of some species of cold-blooded animals, and also that "the sera of some cold-blooded animals contain precipitins." Noguchi states: "It might be said that the existence of normal precipitins for a given serum is more likely to occur in a widely than a closely related species." He assumes that the relationship between the natural precipitins and those produced by adaptation will be found analogous to that which exists between the corresponding natural and artificial agglutinins and hemolysins.

Many observers have noted the fact that the development of adaptation precipitins for the serum of all species is more readily induced in the organism of a species not too closely related. Rabbit serum, according to Lamb,⁶ contains normally a precipitin for cobra venom, and also, according to Obermayer and Pick,⁷ for the dysglobulin of the whites of eggs.

† The culture filtrates which were employed during the course of our investigations, when not otherwise so stated, were obtained from broth made up with Liebig's beef extract, 5 g., 1 per cent. peptone, and 0.5 per cent. sodium chloride, the reaction being approximately 0.5-1 per cent. acid to phenolphthalein. The broth cultures were passed through Berkefeld filters, after an incubation of several months at 37° C. The reaction of our filtrates was alkaline to litmus paper, except that of the diphtheria filtrate, which was acid. The precipitation and agglutination tests were made in sterilized tubes of approximately the same size, and of narrow caliber.

The macroscopic agglutination tests described in this paper were made with suspensions of eighteen-hour agar cultures in 10 c.c. of sterile normal saline solution. After settling for half an hour, 0.9 c.c. of the emulsion, 0.1 c.c. of the serum, or of its dilutions in normal saline solution, was introduced into each tube. Controls were always made.

II. THE PRECIPITATING ACTION OF NORMAL AND ANTIBACTERIAL SERA ON BROTHS OF VARIOUS REACTIONS AND UPON VARIOUS BACTERIAL BROTH FILTRATES.

Normal rabbit and ox serum, as well as antibacterial rabbit sera, were added to broths of various composition and reactions. These broths had a basic composition of 5 g. Liebig's meat extract, 1 per cent. Witte's peptone, 0.5 per cent. NaCl; but varied in their reaction, as follows: 1 per cent. and 0.5 per cent. alkaline, neutral; 0.5 per cent., 1 per cent., and 1.5 per cent. acid, phenolphthalein being the indicator, or were made from the usual meat infusion of different reactions, or of broth made up without additions of pepton.

The tests were made with the following proportions: to 0.5 c.c. of broth was added 0.1 c.c., 0.25 c.c., and 0.5 c.c. of serum. The tests showed that when normal rabbit serum is added to the meat extract broth of 1.5 per cent. acid reaction, small flocculi may develop after twenty-four hours at 37° C. In most cases, however, there is merely a fine granular deposit, which develops on the sides of the tube and is clearly seen only with a low power lens, the fluid remaining clear. This precipitation was never seen in broth of other reactions. In the usual meat infusion, pepton salt broth, normal rabbit serum produces no granular precipitate or flocculi. With normal ox serum no precipitation occurs in broths of different reactions. Normal rabbit and ox serum give rise to opalescence and later to cloudiness, with or without the formation of granules and flocculent precipitates, in broth made up without the usual addition of pepton or salt. The most marked reactions are obtained in broth of 1 per cent. or 1.5 per cent. acid reaction.

In 4 per cent. acid broth a marked turbidity is constantly developed. The reactions obtained in alkaline broth are slight. The nature of the slight precipitates was not determined; in some cases they were possibly small coagula, due to late or uncompleted clotting of the sera, or to the deposition of the cellular elements contained in the sera. Since the reaction of our filtrates was usually alkaline, the cloudiness and precipitates that are developed in broth of 0.4 or 1.5 per cent. acid reaction can be disregarded in our subsequent tests.

The insignificant and late precipitates which are occasionally formed may be disregarded, since they cannot be mistaken for the precipitates developed by the bacterial precipitins upon broth filtrates. The numerous tests made show conclusively that normal rabbit and ox serum, as well as various antibacterial rabbit sera, exert no precipitating action upon the usual peptone salt broth of slight alkaline or acid reaction.

Of especial importance was the observation that normal rabbit serum does not precipitate the various bacterial broth filtrates,* which for the sake

* The broth filtrates of the following species of the colon-typhoid group were tested: *B. typhosus*, *B. coli* "Escherich," *B. psittacosis*, *B. typhi murium*, *B. enteritidis*, *B. icteroides*, *B. Schottmüller* (Seemann and Müller), *B. Gwyn*, *B. Cushing*, and *B. No. 1*, a species of the intermediate group of Durham. The species of the Dysentery group tested were the *B. dysenteriae* Kruse, *B. dysenteriae* "New Haven" (Duval), *B. dysenteriae* "Y" (Hiss). The filtrates of the following bacteria were also tested: *B. proteus*, *B. prodigiosus*, *B. pyocyaneus*, *B. tuberculosis*, human and bovine, *B. diphtheriae*, *Sp. cholera Asiaticae*, *Sp. Metchnikovi*, *Staphylococcus pyogenes aureus*, *Pneumococcus*, and *Streptococcus pyogenes*.

of brevity need not be enumerated here. We are therefore warranted in drawing the conclusion that normal rabbit serum contains no determinable amount of normal bacterial precipitins. The few specimens of ox serum examined likewise indicate the absence of bacterial precipitins in this serum. The question of the presence or absence of normal bacterial precipitins in the serum of other warm- or cold-blooded animals was not determined.

Having demonstrated the absence* of determinable amounts of precipitating substances in the serum of normal rabbits for the filtrates of the different bacterial species which were employed during the course of these studies—an observation in complete accord with those of previous investigators—the writer now purposes to describe a series of observations on the precipitating action of a number of antibacterial sera upon the filtrates of their corresponding and of heterologous species of more or less closely related bacteria: in other words, to test the specificity of the precipitins.

III. THE PRECIPITATING ACTION OF THE SERA OF RABBITS ADAPTED TO VARIOUS SPECIES OF THE COLON-TYPHOID GROUP IN THEIR RESPECTIVE CULTURE FILTRATES.

GENERAL PLAN OF STUDY.

The plan followed in these researches was, first, to study the precipitating action of the serum of rabbits adapted to several distinct species of the so-called colon-typhoid group, in order to determine whether the precipitative relationships exist between various species of this group; and, secondly, to search for more remote relationships, as indicated by slight and late reactions between the bacilli of this and those belonging to other groups of bacilli—namely, *B. proteus*, *B. prodigiosus*—as well as those

* In a strict sense the absence of precipitin haptins for bacteria in normal serum cannot be determined positively, for they may be present in quantities so slight as to escape detection. The writer's observations convince him that the precipitins and agglutinins in antibacterial serum, as a rule, bear the relative proportion, roughly speaking, of 1-100 and that, as a rule, the agglutinin must react in a dilution of 1-100 "macroscopic test" before the presence of a precipitin can be certainly detected. The fact that normal rabbit serum rarely agglutinates bacterial species above 1-100 accounts sufficiently, if the above argument is correct, for the non-detection or absence of the normal precipitins. The writer believes, as will be described later, that this fact cannot be regarded as proof of the identity of those two substances, for an antiserum may precipitate the filtrate of a given species of bacterium without agglutinating the bacterium; and he believes, furthermore, that the precipitins may be formed for a given species without giving rise to any, or only agglutinins capable of reacting in low dilutions.

bacteria belonging to other classes of bacteria, the spirilla and the cocci.

These introductory remarks suffice to indicate the scope of the writer's studies. The first part of our investigations will now be described:

The species used in these series of tests were, first, a bacillus belonging to the hog cholera or intermediate group of Durham, marked B. No. 1; second, a collection culture of *B. typhosus*, designated B. typh. "Coll.;" and, third, a colon bacillus which does not ferment saccharose.*

Sets of rabbits were adapted to each of these species, and the antisera thus obtained were tested, as far as practicable, at the same time, so that their precipitating action upon the same filtrates could be carefully compared. For convenience the results obtained will be described in series.

SERIES I.

ANTI-B. No. 1 RABBIT SERUM.

Rabbit I received 14 c.c. of heated and unheated broth cultures at intervals of several days (November 9, 1901, to January 1, 1902). Bled five days later. The serum agglutinated B. No. 1 up to 1-10,000 (microscopic test); the B. typh. "Coll." to 1-2,000 (microscopic test).

The serum was tested upon the filtrates of the three species which had been grown in broth at 37° C. for twenty-nine days. The filtrates were markedly alkaline to litmus.

TABLE I.

THE PRECIPITATING ACTION OF THE ANTI-B. No. 1 SERUM UPON ITS CORRESPONDING FILTRATE AND UPON THE FILTRATES OF *B. TYPHOSUS* AND *B. COLI*.

B. No. 1 filtrate: 1 c.c.	Anti-B. No. 1 serum: 0.5 c.c.	Tubes kept at 37° C. 2-3 hrs. Cloudiness and flocculent precipitates which soon settle.
B. typh. filtrate: 1 c.c.	Anti-B. No. 1 serum: 0.5 c.c.	Cloudiness is slightly delayed when compared with the homologous filtrate, but at 5 hrs. the reaction is only somewhat less marked. Same phases of reaction.
B. coli filtrate: 1 c.c.	Anti-B. No. 1 serum: 0.5 c.c.	Same phenomenon, only onset of reaction is slower, and the precipitum formed is less than in case of typhoid filtrate.

Tests were then made with similar quantities of the filtrates, but with 0.1 c.c. of serum. With B. No. 1 filtrate the various phases of reaction occurred as rapidly, the precipitum being, however, less abundant; with the typhoid filtrate the reaction was slightly delayed when compared with that

* B. No. 1 and the colon bacillus were isolated from the organs of a patient dying from an infection of five days' duration, clinically a case of severe gastro-enteritis with toxemia.

in the homologous filtrate; a similar, but less marked, reaction was obtained with the colon filtrate.*

To avoid the criticism that we were possibly not dealing with a true typhoid bacillus, the B. No. 1 serum was tested, with identical results, against a filtrate of the well-known typhoid bacillus isolated by Pfeiffer, and known as the "Pfeiffer" culture. On account of the saprophytic condition of the "Pfeiffer" culture, which was only slightly motile, and the rapid pseudo-clumping of its broth cultures and agar suspensions in controls, the agglutinative value of the serum for this bacillus could not be determined.

We thus see from our experiments that the anti-B. No. 1 serum precipitated in dilutions of 1-3 and 1-10, not only the corresponding, but the heterologous, typhoid and colon filtrates; the onset of the reaction cloudiness, however, developing earliest in the homologous filtrate. The amount of precipitum ultimately formed in the B. No. 1 and typhoid filtrates was apparently similar.

ANTITYPHOID RABBIT SERUM.

At intervals of three to six days Rabbit II received subcutaneously ten inoculations of broth cultures, at first heated and then unheated, in all about 25 c.c. Agglutination value, 1-10,000, complete in three hours by macroscopic test; 1-5,000, complete in one hour. The serum did not agglutinate B. No. 1 or the B. coli.

TABLE II.

THE PRECIPITATING ACTION OF THE ANTITYPHOID SERUM UPON ITS CORRESPONDING FILTRATE AND UPON THE FILTRATES OF B. NO. 1 AND B. COLI.

B. typh. filtrate (Coll.): 1 c.c.	Antityph. serum: 0.5 c.c.	At 37° C.: 1 hr., cloudiness; 2 hrs., flocculi; and later, copious precipitate.
B. No. 1 filtrate: 1 c.c.	Antityph. serum: 0.5 c.c.	At 37° C., 2 hrs., cloudiness, flocculi; and later, copious precipitate.
B. coli filtrate: 1 c.c.	Antityph. serum: 0.5 c.c.	No reaction at 2 or 4 hrs.; at 18 hrs., small precipitate.

In a filtrate of a 1 per cent. mannit-broth typhoid culture grown for two weeks at 37° C., having a terminal acid reaction, the onset of the reaction was delayed to twelve hours, and the precipitum formed was less copious than that formed in the B. No. 1 filtrate. With the anti-B. No. 1 serum, this filtrate likewise gave a delayed reaction.

These experiments show, therefore, that the antityphoid serum precipitates its corresponding filtrate and the B. No. 1 filtrate in

*In judging the presence or absence of slight precipitates, to secure accuracy it is imperative to examine the deposit for bacteria; for non-cloudiness in serum mixtures does not signify absence of bacterial growth. When in doubt, tests were made by culture and by examination of coverslips. Usually there is no difficulty in distinguishing between precipitates and bacterial bottom growths, especially if the development of the reaction is watched.

the dilution of 1-3 and 1-5; the reaction being quicker, however, but not more copious, in the homologous filtrate obtained from a broth culture of the same age and terminal reaction. The reaction in the colon filtrate was considerably delayed. That the age and perhaps the terminal reaction of the filtrate exert considerable influence upon the time of onset of the precipitation and the amount of precipitum is shown by the observation that the anti-B. No. 1 serum gives rise to a quicker and more copious reaction in the alkaline typhoid filtrate (four weeks' broth culture at 37° C.) than does the antityphoid serum in the acid typhoid filtrate obtained from a two weeks' mannit-broth culture.

ANTICOLI RABBIT SERUM.

Rabbit III, at intervals of three to five days, received subcutaneous inoculations of heated and unheated broth cultures, in all 24 c.c., one to four days' growth at 37° C. (January 3 to February 10, 1902). Bled several days after last injection. No agglutination of the homologous colon bacillus either by the macroscopic method or by the hanging drop. A slight tendency to thread formation was observed, which ceased in a dilution of 1-1,000. With a serum dilution of 1-10, complete settling occurred only after twenty-four hours at 37° C.

TABLE III.

THE PRECIPITATING ACTION OF THE ANTICOLI SERUM UPON ITS CORRESPONDING FILTRATES AND UPON THE FILTRATES OF B. NO. 1 AND B. TYPHOSUS.

Coli filtrate:	Anticoli serum:	Cloudiness in all tubes in 1 hr. at 37° C., which increases rapidly. 6 hrs., well-marked precipitum, most copious in tube containing 0.25 c.c. serum. The fluid in all the tubes becomes clear.
0.5 c.c.	0.05 c.c.	
0.5	0.10	
0.5	0.15	
0.5	0.25	At 6 hrs., a slight precipitate in the form of fine granules has appeared on the sides of the tubes. At 24 hrs. in the tube containing 0.25 c.c. of serum the precipitum compares in amount to that formed in the homologous filtrate 0.05 c.c. of serum.
B. No. 1 filtrate:	Anticoli serum:	
0.5 c.c.	0.10 c.c.	
0.5	0.25	
B. typh. Coll. filtrate:	Anticoli serum:	Similar reactions to that obtained in B. No. 1 filtrate.
0.5 c.c.	0.10 c.c.	
0.5	0.25	Similar delay in reaction to that obtained with Typh. Coll. filtrate.
B. typh. Pfeiffer fil.:	Anticoli serum:	
0.5 c.c.	0.1 c.c.	
0.5	0.25	

Thus the anticoli serum in dilutions of 1-6 precipitated the corresponding coli filtrate, and also the typhoid and B. No. 1 filtrates. The onset of the reaction in the heterologous filtrates was, however, delayed, and the amount of precipitum formed was considerably smaller than in the colon filtrate.

SUMMARY OF SERIES I.

The results of the first series of tests may be briefly summarized. Precipitative relationships exist between three species of

the colon typhoid group; namely, the intermediate B. No. 1, the B. typhosus, and the B. coli, micro-organisms distinguished from each other by constant biochemical characters of differential value.

ARE PRECIPITINS DEVELOPED IN THE SERUMS OF RABBITS WHICH HAVE ADAPTED THEMSELVES TO INJECTIONS OF PEPTONE?

The rabbits of Series I described above were adapted by means of the usual peptone-broth cultures. Myers,⁸ however, had noted the fact that Witte's peptone gave rise to the formation of substances in the serum of the animals undergoing adaptation which precipitated peptone solutions. This observation led us to suspect that the precipitative relationships noted above between filtrates of various bacterial species, distinct at least as to their biochemical characters, were possibly due alone to the development of a peptone precipitin in the sera of the rabbits of Series I which had been inoculated, as mentioned above, with peptone-broth cultures.

To remove all doubt upon this score, the writer was led to repeat Myers's observations, in order to test his statement that the inoculation of peptone in the animal organism is followed by the formation of substances which precipitate peptone solutions. Our experiments and the literature bearing upon this topic may be briefly reviewed.

Two rabbits were treated with 108 c.c. of a (Witte's) 4 per cent. peptone solution, six intraperitoneal inoculations being made at intervals during four weeks. The sera obtained did not cause even an opalescence in fresh peptone solutions, when added either in equal volumes or in the proportion of 1-5, after twenty-four hours at 37° C. A rabbit treated with large quantities of broth likewise failed to develop precipitating agents in its serum. Intravenous inoculations (five to six inoculations of 0.5 g. of Witte's and Merck's peptone) have likewise proved negative in the hands of my colleagues, Drs. Wood and Jessup, to whom I am indebted for the information.

Myers's observations, although widely quoted and accepted, have likewise not been confirmed by several other investigators. He claims to have obtained precipitins in the serum of rabbits that had adapted themselves to solutions of Witte's peptone. In an article published previous to Myers's, Tchistowitch⁹ states that he had failed to obtain peptone precipitins in the serum of rabbits adapted to peptone. Buchner and Geret¹⁰ cast grave doubts upon the nature of the peptone precipitum obtained by Myers, since it gave no biuret reaction, and, furthermore, because the anti-peptone serum after inactivation by heat was reactivated by normal serum. They endeavored to obtain peptone precipitins by adapting rabbits to peptone, prepared according to Kuhne's method. The rabbits yielded a serum which, when added to a 2 per cent. peptone normal saline solution, gave rise to cloudiness and a fine precipitate composed of so-called globulites, which were found to be crystals of barium sulphate of unusual form.

Rabbits treated with a single injection of 5 c.c. of ox serum after twenty-four hours likewise yielded a serum which developed these crystals.

These observers found that the peptone of commerce invariably contained barium salts. The solutions of peptone freed from the barium salts

were, however, not precipitated by the sera of the rabbits which had been adapted to peptone or to serum.

Michaelis also failed to obtain precipitins in the serum of rabbits which had been treated for six weeks with subcutaneous inoculations of a 10 per cent. solution of Merck's egg peptone (albumose); the receptors which in the whole albumin molecule induce precipitin formation being, Michaelis believes, destroyed by the pepsin digestion to which the egg albumin has been subjected. Similarly with "Peptonum siccum Riedel," which is a mixture of the proteoses derived from the fibrin of ox serum, no precipitating serum was obtained. Although Kowarski obtained precipitins for the albumoses of seeds, Michaelis considers that these albumoses are not comparable to the pepsin albumoses of animal origin, and that, even if in truth albumose and peptone precipitins can be formed, the receptors which would induce the formation of the egg-peptone antibodies cannot be identical to those of egg albumin, for an egg albumin precipitin does not precipitate egg-peptone solutions.¹¹

Obermeyer and Pick¹² have likewise failed to obtain peptone precipitins, although they noted occasionally slight clouding of the peptone solutions by anti-peptone serum. That the question is not finally settled is indicated by the recent investigation of Rostoski and Sacconaghi,¹³ who report that after several failures they succeeded in obtaining antibodies or precipitins in the serum of rabbits which had been inoculated with the peptic and tryptic cleavage products, of horse serum albumin. The sera precipitated the solutions of albumin and those of all the cleavage products, considerable quantitative differences being observed in the reaction, the weakest being obtained in peptone solutions.

Rostoski assumes that the immunizing body, whether it be a special side molecule of the giant albumin molecule, or a body which is merely intimately attached to the albumin molecule, is variously resistant to digestion, according to its association with the different cleavage products. In some cases it is more resistant than the albumin body itself, especially to the action of trypsin.

Ascoli has observed that antisera precipitate the cleavage products of albumin. Thus antihorse and antiox serum of the rabbit precipitate in a slight degree Witte's peptone.* He assumes that digestion destroys some of the groups of the albumin molecule (side chains or receptors of Ehrlich), whereas other groups, those genetically related to the peptone molecule, are left unaffected.

P. A. Levene¹⁴ claims to have shown that a precipitin active for a given proteid is capable of forming precipitates with the primary digestion products of these proteids.

A further proof that the precipitative relationships which had been shown to exist were not due to the formation of precipitins for peptone was furnished by the fact that the antisera of Series I do not precipitate our controls of plain peptone broth, or even 1 per cent. peptone solutions. Nevertheless, to remove all possibility of error, all subsequent rabbits "in this series of studies" were inoculated with emulsions of agar cultures, and not with peptone-broth cultures.

* Possibly this is accounted for by the globulite reaction of Buchner and Geret.

SERIES II.

The tests with the antisera of animals of the first series adapted to the three species above mentioned were preliminary. Those made with the sera of the rabbits of Series II, adapted to the same species, are now given, so far as possible in tabulated form, for brevity of presentation. The rabbits of this series, as well as all subsequent animals, were adapted by inoculations of salt emulsions of agar cultures, grown for twenty-four hours at 37° C. The early inoculations were made with heated and the later with living cultures.

ANTITYPHOID RABBIT SERUM.

Rabbit IV received several inoculations.* Agglutination limit of its serum was found to be 1-1,000 for *B. typh.* "Coll." No action on *B. No. 1* or *B. coli*.

TABLE IV.

THE PRECIPITATING ACTION OF THE ANTITYPHOID SERUM UPON ITS CORRESPONDING FILTRATE AND UPON THE FILTRATES OF *B. No. 1* AND *B. COLI* AND OF VARIOUS OTHER SPECIES OF THE COLON-TYPHOID GROUP.

Typh. fl. (Coll.):†	Antityp. serum			
0.5 c.c.	0.1 c.c.	At 2 hrs. at 37C.: cloudiness.	At 7 hrs.: precipitation has settled out.	At 19 hrs.: scanty precipitum.
0.5	0.25	Fine flocculent precipitate.	At 7 hrs.: precipitation has settled out.	Moderate amount of precipitate.
<i>B. No. 1</i> fl.:				
0.5 c.c.	0.1	Negative.	Negative.	Negative.
0.5	0.25	Negative.	Negative.	Distinct flocculent precipitate.
<i>B. coli</i> fl.:				
0.5 c.c.	0.1	Negative.	Negative.	Small flocculi.
0.5	0.25	Negative.	Negative.	Precipitum.
<i>B. ieteroides</i> fl.:‡				
0.5 c.c.	0.25	At 19 hrs.: negative.	At 43 hrs.: slight cloudiness?	
<i>B. psittacosis</i> fl.:				
0.5 c.c.	0.1	Slight precipitum.		
0.5	0.25	Abundant precipitum.		
<i>B. typhi murium</i> fl.:				
0.5 c.c.	0.1	At 2 hrs.: negative.	At 19 hrs.: small flocculi.	
0.5	0.25	Slight cloudiness.	At 19 hrs.: small precipitate.	
<i>B. dysen. Kruse</i> fl.§				
<i>B. dysen. Flexner</i> fl.:				
0.5 c.c.	0.25		Small precipitate.	
<i>B. enteritidis</i> :				
0.5 c.c.	0.1	At 2 hrs.: negative.	At 7 hrs.: negative.	At 19 hrs.: negative.
0.5	0.25	At 2 hrs.: negative.	At 7 hrs.: negative.	Small flocculi.

* Rabbit IV developed snuffles during adaptation.

† The typhoid, colon, and *B. No. 1* filtrates in these and the subsequent tests were obtained from meat-extract broth cultures grown at 37° C. for ninety-seven days.

‡ The following broth filtrates of same length of incubation at 37° C. were also tested.

§ We are indebted to Professor Flexner for these cultures, which do not ferment mannite, and therefore belong to the true Shiga type, incorrectly called "alkaline" type of *B. dysenteriae*.

A staphylococcus filtrate gave no precipitate after forty-three hours.

It has been shown that, although the agglutinating and precipitating action of the antityphoid serum was considerably weaker than that of the typhoid antiserum of the first series, nevertheless the serum precipitated the filtrates of all the colon-typhoid group tested, the negative result obtained with the filtrate *B. icteroides* being most probably due to the scanty growth of the bacillus in the broth from which the filtrate was obtained.

ANTI-B. No. 1 SERUM.

Rabbit V between March 19 and April 2, 1902, received five agar cultures; bled April 18. The following microscopic agglutination tests were made with the serum upon B. No. 1:

1-20,000: nearly complete in $\frac{3}{4}$ hr.; complete in 3 hrs. at 37° C.; and 1-40,000, complete at 6 hrs.

The serum tested upon the typhoid bacillus gave the following reaction:

1-100: at $\frac{1}{2}$ hr. reaction begins, large clumps, but fluid still cloudy.

1-1,000: at 3 $\frac{1}{2}$ hrs., large clumps; tube is nearly clear.

1-10,000: negative at 3 $\frac{1}{2}$ hrs.; at 5 hrs., small clumps. At 5 hrs. none of the tubes are, however, perfectly clear.

TABLE V.

THE PRECIPITATING ACTION OF THE ANTI-B. No. 1 SERUM UPON ITS CORRESPONDING FILTRATE AND UPON THE FILTRATES OF *B. TYPHOSUS*, *B. COLI*, AND OF VARIOUS OTHER SPECIES OF THE COLON-TYPHOID GROUP.

B. No. 1 filtrate:	Anti-B. No. 1 serum:	
0.5 c.c.	0.10 c.c.	Instant cloudiness.
0.5	0.25	Instant cloudiness; precipitum at room temperature forms in less than $\frac{1}{2}$ hr.
B. typh. filtrate:		
0.5 c.c.	0.10	Instant cloudiness; at 1 $\frac{1}{2}$ hrs., fine precipitum.
0.5	0.25	Instant cloudiness; at 1 $\frac{1}{2}$ hrs., fine precipitum; at 6 hrs., flocculent precipitum.
B. colon filtrate:		
0.5 c.c.	0.10	After several hours, cloudiness.
0.5	0.25	At 1 $\frac{1}{2}$ hrs., cloudiness; at 6 hrs., a finely flocculent precipitate.
B. psittacosis:		
0.5 c.c.	0.10	Immediate cloudiness; reaction in both tubes complete at 6 hrs.
0.5	0.25	
B. typhi murium:		
0.5 c.c.	0.10	As above.
0.5	0.25	
B. enteritidis:		
0.5 c.c.	0.25	Cloudiness at 1 $\frac{1}{2}$ hrs.; precipitate at 6 hrs.
0.5	0.10	Cloudiness at 1 $\frac{1}{2}$ hrs.; none at 6 hrs., but slight precipitate at 18 hrs.
B. icteroides:		
0.5 c.c.	0.25	Both tubes show slight precipitate at 19 hrs.
0.5	0.25	
B. dysenteriae— Flexner and Kruse:		
0.5 c.c.	0.25	No cloudiness at 6 hrs.
0.5	0.1	At 19 hrs., slight flocculi in both tubes.
Staphylococcus filtrate:		No reaction after 72 hrs.

In the hanging drop, the following reactions were obtained with the typhoid bacillus:

1-20: complete clumping at 1 hr.; also at 24 hrs.

1-200: complete reaction; clumps are small, but slightly motile. No free bacilli.

1-20,000: at $\frac{3}{4}$ hr. small motile clumps; free motile bacilli.

These agglutinative relationships are of considerable interest, especially in connection with those we have demonstrated to exist in the case of the precipitins.*

The onset of reaction and amount of precipitum developed in the filtrates of *B. psittacosis*,† and *typhi murium* and the homologous *B. No. 1* filtrate were similar, the typhoid filtrate perhaps giving a less copious precipitum. With *B. enteritidis* the reaction was less marked, although the addition of 0.25 c.c. of serum to 0.5 c.c. of filtrate gave rise to a copious precipitum at six hours. The filtrates of the dysentery bacilli gave a flocculent precipitate at nineteen hours with 0.1 c.c. and 0.25 c.c. of serum. It has thus been demonstrated with anti-*B. No. 1* that a serum adapted to one species of the colon-typhoid group precipitates, in dilutions of 1-6, the filtrates of many different species of this group.

*Castellani and Durham, Theobald Smith, H. Bruns and H. Kayser,¹⁵ and many others, have recorded numerous examples of agglutinative affinities between the members of the colon-typhoid group. Carl Sternberg has shown that antityphoid serum may agglutinate, in dilutions of 1-5,000, several examples of so-called paratyphoid bacilli obtained from potable waters. The interreactions described above between the typhoid bacillus and the anti-*B. No. 1* serum do not always occur, as shown later on. No antityphoid rabbit serum has been obtained which agglutinated the *B. No. 1*, except in 1-10 dilutions, although such sera frequently gave rise to more copious precipitates in the *B. No. 1* filtrates than did some of the weaker anti-*B. No. 1* sera, especially when tested in dilutions of 1-10 or 1-5. This statement does not apply to the reactions obtained with higher dilutions of serum, the homologous serum always precipitating its corresponding filtrate in higher dilution than does the heterologous serum.

†The *B. psittacosis* and *B. typhi murium*, both obtained from Král, cannot be differentiated from our *B. No. 1* by cultural tests. In fact, the *B. psittacosis* is agglutinated and its filtrate precipitated by the anti-*B. No. 1* serum quite as effectively as the homologous bacillus, in some tests even more so. The *B. typhi murium*, however, does not agglutinate in such high dilutions or furnish quite as copious precipitates as does the *B. No. 1*. Nevertheless, as detailed later on, this was determined only by a careful estimate of the maximal serum dilutions which determine precipitation. Whether an animal immunized against *B. No. 1* is also protected against these two bacilli of the intermediate group was not determined, although it seems most probable. The *B. enteritidis* is not agglutinated by the anti-*B. No. 1* serum at all; by the antityphoid sera often in dilutions of 1-100 and 1-200. (Durham has made similar observations upon the action of antityphoid serum on *B. enteritidis*.)

ANTICOLI SERUM.

Rabbit VI, between March 1 and April 2, 1902, received seven agar cultures; bled April 7.

TABLE V.

THE PRECIPITATING ACTION OF THE ANTICOLI SERUM UPON ITS CORRESPONDING FILTRATE AND UPON THE FILTRATES OF B. NO. 1 AND B. TYPHOSUS, AND OF VARIOUS OTHER SPECIES OF THE COLON-TYPHOID GROUP.

Colon fil.: 0.5 c.c.	Anticoli ser.: 0.1 c.c.	Immediate cloudiness; at 1 hr., flocculi.	At 5½ hrs., flocculi still floating.	At 19 hrs., complete.
0.5	0.25	Immediate cloudiness; at 1 hr., flocculi.	Reaction complete.	
B. typhi murium fil.:				
0.5 c.c.	0.10	At 1 hr., negative.	At 5½ hrs., cloudiness?	At 19 hrs., moderate flocculent precipi- tum in both tubes.
0.5	0.25			
B. No. 1 fil.:				
0.5 c.c.	0.10	Cloudiness?	Slight cloudi- ness in 0.25 c.c.	In 0.1 c.c., flocculent precipitum less abundant than ho- mologous tube.
0.5	0.25			
B. psittacosis fil.:				
0.5 c.c.	0.10	Cloudiness?	Marked cloudi- ness.	In 0.1 c.c., cloudiness; 0.25 c.c., marked cloudiness.
0.5	0.25			
B. typhoid fil.:				
0.5 c.c.	0.10	Cloudiness?	Marked cloudi- ness.	Both tubes show moderate amount of precipitum.
0.5	0.25			
B. enteritidis fil.:				
0.5 c.c.	0.10	Negative.	Slight cloudi- ness in both.	Both tubes show moderate amount of precipitum.
0.5	0.25			
B. icteroides fil.:				
0.5 c.c.	0.10	At 1 hr., negative.	At 5½ hrs., negative.	At 19 hrs., negative.
0.5	0.25			
B. dysenteriae fil., Kruse and Flex- ner:				
0.5 c.c.	0.10	At 1 hr., negative.	At 5½ hrs., negative.	Very slight reaction in both tubes.
0.5	0.25			
Staphylococcus fil.:				
0.5 c.c.	0.10	At 1 hr., negative.	At 5½ hrs., negative.	In 0.25 c.c. tube, a slight precipitate on sides.
0.5	0.25			

The anticoli serum thus causes precipitation, not only in the filtrates of bacilli belonging to the intermediate group, but also in the filtrates of the dysentery bacillus.

The anticoli serum thus precipitated the filtrates of various and distinct species of the intermediate and dysentery groups, except that of *B. icteroides* in dilutions of 1-5. Upon the staphylococcus filtrate it has no action.

SUMMARY OF SERIES II.

The results of the tests of Series II may be summarized briefly. The sera of three rabbits, immunized respectively to *B. typhosus*, the intermediate *B. No. 1*, and *B. coli*, precipitated in dilutions of 1-3 and also 1-6, not only their homologous, but also a large number of the filtrates of heterologous, species belonging to the same so-called colon-typhoid group. Precipitative relationships between the species of the same group are thus indicated.

SERIES III.

In order to confirm the observations made with the antisera of the rabbits of Series I and II, and also to enable us to widen the scope of our investigations, a third set of rabbits was adapted to the species of the colon-typhoid group, namely, *B. No. 1*, *B. typhosus*, and *B. coli*.

ANTI-B. No. 1 SERUM.

Rabbit VII received six agar cultures, between April 4 to May 26, 1902; bled June 1. Agglutinating value: 1-5,000 to 1-10,000. No agglutinating action upon the typhoid or colon bacillus.

TABLE VI.

THE PRECIPITATING ACTION OF THE ANTI-B. No. 1 SERUM UPON ITS CORRESPONDING FILTRATE AND UPON THE FILTRATES OF *B. TYPHOSUS* AND *B. COLI*.

B. No. 1 filtrate: 0.5 c.c. 0.5	Anti-B. No. 1 serum: 0.25 c.c. 0.10	At ½ hr., slight cloudiness; at 4 hrs., fine precipitation; at 10 hrs., copious precipitation in both tubes, fluid being clear and sterile.
B. typh. filtrate: 0.5 c.c. 0.5	0.25 0.10	At ¾ hrs., slight cloudiness; at 4 hrs., not as marked as in homologous filtrate; at 10 hrs., the amount of precipitum is similar to above set.
B. coli filtrate 0.5 c.c. 0.5	0.25 0.10	No reaction at 4 hrs.; very slight reaction in both tubes at 24 hrs.; at 48 hrs., the tube with the larger amount of serum contains a copious precipitum.

ANTI-B. TYPHOID SERUM.

Rabbit VIII received, from April 10 to June 12, 1902, nine twenty-four-hour old agar cultures; bled June 17. Agglutinating value between 1-5,000 and 1-10,000. No agglutinating reaction upon *B. No. 1* (1-10, complete in four hours; 1-100, negative).

TABLE VII.

THE PRECIPITATING ACTION OF THE ANTITYPHOID SERUM UPON ITS CORRESPONDING FILTRATE AND UPON THE FILTRATES OF B. NO. 1 AND B. COLI.

Typhoid filtrate: 0.5 c.c. 0.5	Antityph. serum: 0.25 c.c. 0.10	Cloudiness in several minutes in both tubes; at 1 hr., small flocculi; at 4 hrs., copious precipitum, fluid clear; at 29 hrs., amount of precipitum similar in both tubes.
B. No. 1 filtrate: 0.5 c.c. 0.5	0.25 0.10	At 1 hr., opalescence and cloudiness; at 4 hrs., precipitation not quite as marked as with the homologous filtrate; at 29 hrs., the two sets of tubes are similar.
B. coli filtrate: 0.5 c.c. 0.5	0.25 0.10	Negative at 4 hrs.; at 19 hrs., large flocculi in the 0.25 c.c. serum tube. In the 0.10 c.c. tube scanty precipitum at 48 hrs. The reaction was slower and much less copious than with the other filtrates.

The tests with fresh anticoli serum could not be made owing to the death of the rabbits during the course of adaptation.

The tests made with the antisera of rabbits of Series III confirm the previous observations of Series I and II in regard to the precipitating action of the serum of a rabbit adapted to one species, upon the filtrates of two other species of the colon-typhoid group.

RECORD OF FURTHER TESTS MADE WITH THE ANTISERA OF SERIES II AND III, WITH THE SAME AND WITH ADDITIONAL FILTRATES.

The precipitating action of the antisera of the rabbits of Series II and III adapted to the three species of the colon group upon filtrates of other species of this same group which have not as yet been referred to, will now be given, for brevity, in tabulated form, the sera of Series II having been kept two months; that of Series III, several weeks.

SUMMARY.

Table VIII indicates that a highly active antiserum, "anti-B. No. 1," induces precipitation in a large number of heterologous filtrates (even after preservation for two months in cold storage). On the other hand, an antiserum, "antityphoid," originally much less active than the above anti-B. No. 1 serum, as indicated by its low agglutination value, 1-1,000, possesses only a slight precipitating action upon its corresponding and none upon heterologous filtrates.

TABLE VIII.

THE PRECIPITATING ACTION OF ANTI-B. NO. 1 AND ANTI-B. TYPHOSUS SERA UPON THEIR CORRESPONDING FILTRATES AND UPON THE FILTRATES OF HETEROLOGOUS SPECIES OF THE COLON-TYPHOID GROUP.

(Test made June 18, 1902.)

Filtrate	Anti-B. No. 1 Serum. Rabbit V, Series II. Agglutinating Value, 1-20,000. Bled April 8, 1902	Antityphoid Serum. Rabbit IV, Series II. Agglutinating Value, 1-1,000. Bled, April 2, 1902
0.5 c.c. B. coli: 1/3-4/10/1902. B. typhosus: 1/3-4/10/1902.	0.25 c.c. Negative at 48 hrs. Cloudiness at 4 hrs.; slight precipitum at 18 hrs.; more marked at 48 hrs.	0.25 c.c. Negative at 48 hrs. Negative at 18 hrs.; slight precipitum at 48 hrs.
B. No. 1: 1/3-4/10/1902.	Cloudiness at 1 hr.; slight precipitum at 4 hrs.; abundant precipitum at 18 hrs.	Slight precipitum at 48 hrs.
B. Schottmüller:* Stamm Seemann: 4/11-6/6/1902. Stamm Müller: 4/11-6/6/1902.	Cloudiness at 1 hr.; well-marked precipitum at 4 hrs. Cloudiness at 4 hrs.; slight precipitum at 48 hrs.	Negative at 48 hrs. Negative at 48 hrs.
B. Gwyn: 4/11-6/4/1902.	Cloudiness at 1 hr.; well-marked precipitum at 4 hrs.	Negative at 48 hrs.
B. Cushing: 4/11-6/4/1902.	Opalescence at 4 hrs.; distinct precipitum at 18 hrs.	Negative at 48 hrs.

TABLE IX.

THE PRECIPITATING ACTION OF ANTI-B. NO. 1, ANTI-B. TYPHOSUS, AND ANTI-B. COLI SERA UPON THEIR CORRESPONDING FILTRATES AND UPON THE FILTRATES OF HETEROLOGOUS SPECIES OF THE COLON-TYPHOID GROUP.

(Test made June 21, 1902.)

Filtrate	Anti-B. No. 1 Serum. Rabbit VIII, Series III. Agglutinating Value, 1-5-10,000. Bled June 1, 1902	Antityphoid Serum. Rabbit VII, Series III. Agglutinating Value, 1-5-10,000. Bled June 17, 1902	Anticoli Serum. Rabbit VI, Series II. No Agglutinating Value. Bled April 7, 1902
0.5 c.c. B. Schottmüller: Seemann.	0.1 c.c. At ½ hr., cloudiness; at 19 hrs., copious precipitum.	0.1 c.c. At ½ hr., cloudiness; at 19 hrs., copious precipitum.	0.1 c.c. Slight precipitum at 48 hrs.
Müller.	Negative at 4 hrs.; slight cloudiness and precipitum at 19 hrs.	Negative at 4 hrs.; slight precipitum at 19 hrs.	Negative at 4 hrs.; slight precipitum at 19 hrs.
B. Gwyn.	Slight precipitum at 19 hrs.	Slight precipitum at 19 hrs.	Negative at 48 hrs.
B. Cushing.	Cloudiness at ½ hr.; marked precipitum at 4 hrs.; copious precipitum at 18 hrs.	Cloudiness at ½ hr.; marked precipitum at 4 hrs.; copious precipitum at 18 hrs.	Small amount of precipitum at 24 hrs.
B. psittacosis.	Cloudiness at ½ hr.; copious precipitum at 4 hrs.; most marked reaction of all.	Cloudiness at ½ hr.; copious precipitum at 4 hrs.	Same as above.
B. typhi murium. B. icteroides.	Same as above. Negative at 4 hrs.; small amount of precipitum at 19 hrs.	Same as above. Negative at 4 hrs.; at 19 hrs., small amount of precipitum.	Same as above. Negative at 48 hrs.
B. enteritidis.	Cloudiness at ½ hr.; moderate amount of precipitum at 4 hrs.	Moderate amount of precipitum at 19 hrs.	Slight amount of precipitum at 24 hrs.

*The filtrates of Schottmüller, Gwyn, and Cushing were obtained from meat-infusion broth. The writer is personally indebted to Dr. Schottmüller for his cultures.

The filtrates psittacosis, typhi murium, icteroides, and enteritidis, were alkaline to litmus paper, and were obtained from Liebig's meat-extract peptone-salt broth, grown for three months at 37° C. and then passed through Berkefeld filters.

SUMMARY.

Table IX demonstrates the fact again that highly active antisera adapted to one species precipitate the heterologous filtrates of various species belonging to the same group. The anticolon serum which had been kept for seventy-five days produced no reaction, or only slight precipitates, in the heterologous filtrates. It must be borne in mind that, even when fresh, this anticolon serum did not produce copious precipitates in its corresponding filtrate.

The Müller, Gwyn, and icteroides filtrates gave the scantiest reactions. The most copious precipitates were furnished by the Seemann, psittacosis, and typhi murium filtrates, which reacted with the antityphoid and anti-B. No. 1 sera in serum dilutions of 1-6 as copiously as did the antisera in their corresponding filtrates. The onset of reaction, cloudiness, is practically instantaneous in the corresponding filtrates, whereas in the heterologous filtrates it is delayed for fifteen to twenty minutes or more.

A STUDY OF THE PRECIPITATING VALUE OF SERA ADAPTED TO SPECIES OF THE COLON-TYPHOID GROUP FOR THEIR HOMOLOGOUS AND HETEROLOGOUS CULTURE-BROTH FILTRATES. A DETERMINATION OF THE MAXIMAL LIMITS OF PRECIPITATION OF ANTISERA FOR THEIR CORRESPONDING AND HETEROLOGOUS FILTRATES.

In order to determine the limits of the precipitating action of antisera for their corresponding and heterologous filtrates, the following series of tests were made, at first in serum dilutions of 1-50 (5 c.c. of filtrate and 0.1 c.c. of serum), November 8, 1902:

TABLE X.

THE PRECIPITIN REACTIONS OBTAINED WITH ANTI-B. NO. 1, AND ANTITYPHOID SERUM DILUTIONS OF 1-50, IN THEIR CORRESPONDING FILTRATES AND IN THE FILTRATES OF HETEROLOGOUS SPECIES OF THE COLON-TYPHOID GROUP.

Filtrate	Anti-B. No. 1 Serum. Agglutinating Value, 1-10,000. Bled Oct. 24	Antityphoid Serum. Agglutinating Value, 1-20,000. Bled Oct. 24
B. typh. 5 c.c.	0.1 c.c. Negative up to 24 hrs.	0.1 c.c. At ½ hr., slight cloudiness; at 2½ hrs., much cloudiness; at 22 hrs., abundant precipitate.
B. No. 1.	Slight cloudiness at ½ hr.; at 2½ hrs., much cloudiness; at 22 hrs., heavy precipitate.	Negative up to 4 and 24 hrs.
B. coli.	Negative up to 22 hrs. and later.	Negative up to 22 hrs.; at 48 hrs., a faint cloudiness.
B. typh., Pfeiffer.	Negative at 4 and 24 hrs.	Identical reaction to that given by the typhoid filtrate above.
B. dysenteriae: "Flexner." "Kruse." "Seward."	Negative up to 4 and 24 hrs. Negative up to 4 and 24 hrs. Negative up to 4 and 24 hrs.	At 22 hrs., slight cloudiness. At 22 hrs., slight cloudiness. At 22 hrs., slight cloudiness.
B. Cushing.	Negative at 2½ hrs.; at 22 hrs., moderate amount of precipitate.	No precipitate develops. Negative at 2½ hrs., at 48 hrs., and later.
B. Gwyn.	Negative up to 22 hrs.; at 48 hrs., small amount of precipitate.	Negative up to 4 and 24 hrs.*
B. Schottmüller; "Seemann."	Negative at 2 hrs.; small amount precipitum at 22 hrs.; at 48 hrs., considerable precipitum.	Negative at 22 hrs. and later.
"Müller."	Negative at 22 hrs.; perhaps slight cloudiness and small amount of precipitate at 48 hrs.	Negative at 48 hrs.
B. psittacosis.	Slight cloudiness at ½ hr., which has increased in amount at 2½ hrs.; at 22 hrs., a copious precipitate.	At 2½ hrs., slight cloudiness; at 22 hrs., cloudiness and small amount of precipitate, which at 48 hrs. has increased.
B. typhi murium.	At 2½ hrs., very slight cloudiness; at 22 hrs., distinct and moderate amount of precipitate; more at 48 hrs.	Negative at 48 hrs. and later.
B. enteritidis.	Negative at 48 hrs. and later.	Slight cloudiness at 22 hrs.; no precipitate develops.
B. icteroides.	Negative at 22 hrs.; slight amount of precipitate at 48 hrs.	Negative at 22 hrs.; at 48 hrs. ?
B. suipestis.	Negative at 48 hrs. and later.	Negative at 48 hrs.

* Addition of 0.1 c.c. of both sera together gave copious precipitate at twenty-two hours. All filtrates strongly alkaline to litmus paper.

SUMMARY.

The table indicates that the sera of rabbits adapted to one species of the colon-typhoid group precipitates in dilutions of 1-50, the filtrate of many, but not all, of the species in this group.

DETERMINATION OF THE MAXIMAL PRECIPITATION LIMITS OF THE ANTI-B.
No. 1 SERUM FOR ITS HOMOLOGOUS BACILLUS.

In order to determine the precipitation limits of the anti-B. No. 1 serum more accurately than was indicated by the above table, the following tests were made:

To each tube was added 0.9 c.c. of filtrate and 0.1 c.c. of serum, or its dilutions in sterile 0.85 per cent. saline solution.

TABLE XI.
ANTI-B. No. 1 SERUM; AGGLUTINATION LIMIT, 1-10,000.

B. No. 1 filtrate:	B. No. 1 serum:	
9/10 c.c.	1/20 c.c.	Cloudiness marked at 1 hr.
9/10	1/40	Faint cloudiness at 1 hr.
9/10	1/60	Faint cloudiness at 1 hr.
9/10	1/80	Faint cloudiness at 1 hr.
9/10	1/100	Negative at 1 hr.; negative at 48 hrs.
9/10	1/120	Negative at 1 hr.
9/10	1/150	Negative at 1 hr.
		At 20 hrs., 1/100 c.c., a very faint reaction;
		1/150 c.c., reaction is possibly present; at
		48 hrs., a distinct small flocculus had
		formed. Reaction thus occurred at 1-1,500.

The limit of the precipitating action of the anti-B. No. 1 serum for the homologous filtrate was thus shown to be 1-150. By a similar method the limits of the action of the serum upon the following filtrates of broth cultures of approximately similar lengths of incubation were found:*

- B. psittacosis, 1-150.
- B. typhi murium, 1-120.
- B. Schottmüller (Seemann), and B. Cushing, 1-80.
- B. Gwyn, 1-40.
- B. enteritidis, 1-50.

The serum did not precipitate in a dilution of 1-50, the filtrates B. typhosus, B. coli, B. dysenteriae, "Flexner," "Kruse," and "New Haven" (non-mannite fractors) or B. icteroides. The B. Schottmüller (Müller) gave a questionable reaction with the above serum dilution.

DETERMINATION OF THE MAXIMAL LIMITS OF THE ANTI-B. TYPHOID SERUM
FOR ITS HOMOLOGOUS BACILLUS.

ANTITYPHOID SERUM.

Agglutination limit, 1-20,000.

The serum was tested as was the anti-B. No. 1 serum, as above described. The precipitation limit for the homologous typhoid filtrate was found to be 1-150; for the psittacosis, 1-140; the filtrates of three dysentery races and

*Two factors, it may here be stated, were always taken into account in determining the precipitating value of an antibacterial serum: the degree of adaptation of the animal to the bacterial species, and the age and abundance of the growth of the broth culture from which the filtrate has been obtained. In the series of tests which were made to determine the limits of the precipitating action of the antisera upon their corresponding and heterologous filtrates, the various filtrates were accordingly obtained from broth cultures of approximately the same age.

B. enteritidis, 1-50 (slight cloudiness), no reaction in the other filtrates being obtained with a serum dilution of 1-50.

THE PRECIPITIN REACTIONS OBTAINED WITH A FRESH ANTICOLI SERUM.

ANTICOLI SERUM.

Agglutination limit, 1-250.

Owing to unavoidable circumstances, no anticoli rabbit serum was at hand at the time the above tests were made. A rabbit which had received eight and a half agar cultures during a period of fifty-one days, however, furnished a serum of high precipitative powers. The serum agglutinated* the homologous colon bacillus completely in a dilution of 1-250 in seven hours. Another colon bacillus of the same type (non-saccharose splitter) agglutinated only in a dilution of 1-100. The *B. typhosus* "Coll." and the intermediates were not agglutinated in dilutions above 1-10 and 1-50, respectively.

DETERMINATION OF THE MAXIMAL PRECIPITATION LIMITS OF THE ANTICOLI SERUM FOR ITS HOMOLOGOUS FILTRATE.

The following tests were made to determine the precipitating limit, that is, the maximal dilution, at which a reaction is obtained by the anticoli serum in its homologous filtrate:

Each tube contained 5 c.c. of filtrate, and to each was added 1/10 c.c. of the diluted serum.

TABLE XII.

Filtrate	Anticoli Serum	2 Hrs.	6 Hrs.	24 Hrs.	72 Hrs.
5 c.c.	1/25 c.c.	Negative.	Very slight.	Clear fluid with slight precipitate.	Fairly abundant precipitate.
5	1/50	Negative.	Negative.	Negative.	Clear, sterile fluid; slight precipitate.
5	1/100	Negative.	Negative.	Negative.	Clear fluid; slight, but typical precipitate.
5	1/250	Negative.	Negative.	Negative.	Negative.

The maximal dilution at which a reaction is obtained was thus found to be about 1-500.

THE PRECIPITIN REACTIONS OBTAINED WITH ANTICOLI SERUM DILUTIONS OF 1-50 IN ITS CORRESPONDING FILTRATE AND IN THE FILTRATES OF HETEROLOGOUS SPECIES OF THE COLON-TYPHOID GROUP.

The anticoli serum was next tested upon the following filtrates:

Each tube contained 5 c.c. of filtrate and 1/10 c.c. of the serum. Tests were made at 37° C.

*The low agglutinative value of the serum for the *B. coli* the writer does not believe can be ascribed to the recent isolation of the bacillus from the cadaver, for it had been cultivated for a year, about twelve generations; and, furthermore, a stock colon bacillus was likewise agglutinated by the serum only in low dilutions. It is a well-known fact that freshly isolated cultures which themselves are more or less inagglutinable do give rise to abundant production of immune bodies in animals. The inagglutinability of typhoid and pyocyanus cultures of recent isolation has been described in EISENBERG's interesting article, to which the reader is referred.¹⁶

TABLE XIII.

Filtrate 5 c.c.	3 Hrs.	6 Hrs.	8½ Hrs.	12 Hrs.	24 Hrs.
B. Schottmüller:					
Stamm Müller	Negative.	Negative.	Negative.	Negative.	Negative.
Stamm Seemann	Negative.	Negative.	Negative.	Negative.	Negative.
B. enteritidis	Very slight cloudiness.	Slight.	Slight.	Slight cloudiness; no precipitate.
B. psittacosis	Negative.	Negative.	Negative.	Slight.	Considerable precipitum; fluid clear.
B. icteroides	Very slight cloudiness.	Slight.	Slight.	Considerable precipitum; fluid clear.
B. typhi murium	Negative.	Negative.	Negative.	Negative.	Slight precipitum
B. typhi	Negative.	Negative.	Slight.	Slight.	Slight precipitum
B. No. 1	Negative.	Negative.	Negative.	Negative.	Slight precipitum
B. dysenteriae:					
"Flexner"	Very slight cloudiness?	Very slight cloudiness.	Slight precipitum; fluid nearly clear.
"Kruse"	Very slight cloudiness.	Slight.	Cloudiness, quite marked	Slight precipitate; fluid clear.
"Flexner" (New Haven)	Very slight cloudiness.	Slight.	Slight.	Considerable precipitate.
B. Gwyn	Negative.	Negative.	Negative.	Negative.
B. Cushing	Negative.	Negative.	Negative.	Considerable precipitate; fluid clear.
B. proteus*	Negative.	Negative.	Negative.	Negative.
Sp. Metchnikovi	Negative.	Negative.	Negative.	Negative.
Sp. cholerae	Negative.	Negative.	Negative.	Negative; precipitate?
Staphylococcus pyogenes aureus	Negative.	Negative.	Negative.	Negative.

B. coli (homologous) clouding within fifteen minutes; at six hours, slight precipitation and marked clouding.

The filtrates of the three dysentery races have nearly as copious precipitate at twenty-four hours as the colon. At seventy-two hours precipitate have increased in amount, in *B. No. 1*, Seemann, typhoid, icteroides, psittacosis (copious), typhi murium, enteritidis, and Müller (very slight); Cushing, quite copious; Gwyn, less so; staphylococcus, *Sp. Metchnikovi*, and *B. proteus*, filtrates absolutely negative.

The anticoli serum, we have already shown, gives a reaction in the colon filtrate in a dilution of 1-500. In a dilution of 1-50 the serum precipitates the filtrates of *B. enteritidis*, psittacosis, icteroides, typhi murium, *B. typhosus*, *B. No. 1*, *B. dysenteriae*, Flexner, Kruse, and New Haven (non-mannite fractors), and *B. Cushing*, after twenty-four hours at 37° C., *B. Gwyn* giving only a slight reaction. The filtrates of other bacterial groups, such as *B. proteus*, *B. prodigiosus*, the vibrios, cholerae and Metchnikovi, and the staphylococcus, are not acted upon.

* Referred to later on.

THE PRECIPITIN REACTIONS OBTAINED WITH ANTICOLI SERUM DILUTIONS OF 1-10 IN ITS CORRESPONDING FILTRATE AND IN THE FILTRATES OF HETEROLOGOUS SPECIES OF THE COLON-TYPHOID GROUP.

Each tube contains 1 c.c. of filtrate and 1/10 c.c. of anticoli serum.

TABLE XIV.

	15-20 Minutes	1¼ Hour	18 Hours.	72 Hours.
Schottmüller: Seemann	Negative.	Cloudiness.	Like typhoid, but a little less.	
B. enteritidis	Cloudiness.	Marked cloudi- ness.	Same as above.	
B. typhi murium	Cloudiness.	Marked cloudi- ness.	Heavy precipitum, like B. No. 1.	
B. Gwyn	Cloudiness.	Cloudiness.	Less than enteritidis.	
B. Cushing	Cloudiness.	Cloudiness.	Less than enteritidis.	
B. psittacosis	Cloudiness.	Cloudiness.	About like B. No. 1.	
B. No. 1	Marked cloudi- ness.	Less than colon.	Like colon tube, only slightly less in bulk. Next to colon tube.	
B. typhi (collec- tion)	Marked cloudi- ness.	Less than colon.	Like B. No. 1, perhaps less.	
B. typhi Pfeiffer	Cloudiness.	Marked cloudi- ness.	Less than other typhoid fil- trate.	
B. coli	Most marked cloudiness of all.	Marked precipitum on sides, which settles out on agi- tating tube.	
B. icteroides	Negative.	Negative.	Very small amount; increased at 72 hrs.	
B. dysenteriae: "Flexner"	Slight cloudi- ness.	Marked cloudi- ness, but less than B. No. 1 and B. typh. (col.).	Flocculent precipita.	
"Kruse"	Slight cloudi- ness.	Flocculi.		
"New Haven"	Slight cloudi- ness.	Flocculi.		
Sp. Metchni- kovi	Negative.	Negative.	Negative.	Negative.
Sp. cholerae	Negative.	Negative.	Negative.	Negative.
Streptococcus pyogenes aureus	Negative.	Negative.	Negative.	Negative.
B. prodigiosus	Negative.	Negative.	Negative.	Negative.

With one to ten anticoli serum dilutions, after eighteen hours, little difference in the amount of precipitum formed is made out between the colon and typhoid, B. No. 1, B. enteritidis, B. typhi murium, and B. psittacosis filtrates. The onset of the reaction with colon filtrate is, however, immediate, whereas in the typhoid and B. No. 1 filtrates the cloudiness reaction appears after fifteen minutes. At seventy-two hours all tubes containing filtrates of the colon-typhoid group contain a considerable amount of precipitum. The icteroides filtrate contains the least; but this is accounted for by the fact that the growth obtained in the broth culture which furnished the filtrate was considerably less than

those of the other bacilli, with the possible exception of the *B. dysenteriae* "Flexner" filtrate, in which the amount of precipitum was also small.*

SUMMARY OR TABULATION OF THE MAXIMAL LIMITS OF PRECIPITATION
OBTAINED WITH THE THREE FRESH ANTISERA.

The maximal limits of precipitation of the three antisera in the various filtrates, so far as determined, are represented in the following table:

TABLE XV.

		Limit of Pre- cipitation
B. No. 1 filtrate.....	October 24: Anti-B. No. 1 serum — aggluti- nation limit, 1-10,000	1-150 1-150 1-120-150
B. psittacosis filtrate.....	"	1- 80
B. typhi murium filtrate.....	"	1- 80-100
B. Schottmüller, "Seemann" filtrate.....	"	1- 40
B. Cushing filtrate.....	"	1- 50
B. Gwyn filtrate.....	"	
B. enteritidis filtrate.....	"	
B. typh. filtrate.....	October 24: Antityphoid serum — aggluti- nation limit, 1-20,000	1-150 1-140
B. psittacosis filtrate.....	"	
B. dysenteriae, Flexner, Kruse and New Haven filtrate.....	"	1- 50
B. colon filtrate.....	November 25: Anti-colon serum — agglutina- tion limit, 1-250	1-500
B. enteritidis filtrate.....	"	1- 50
B. psittacosis filtrate.....	"	1- 50
B. icteroides filtrate.....	"	1- 50
B. typhi murium filtrate.....	"	1- 50
B. typhoid filtrate.....	"	1- 50
B. No. 1 filtrate.....	"	1- 50
B. Cushing filtrate.....	"	1- 50
B. dysenteriae, three races, filtrate.....	"	1- 50

IV. THE PRECIPITATING ACTION OF THE SERA OF RABBITS
ADAPTED TO VARIOUS RACES OF THE *B. DYSENTERIAE*
SHIGA, AND ALSO TO OTHER SPECIES OF THE SO-CALLED
DYSENTERY GROUP, WHICH FERMENT MANNITE, IN THEIR
RESPECTIVE CULTURE FILTRATES.

The tests made with antidysenteric rabbit sera are now to be described, for although the *B. dysenteriae* doubtless belongs to the colon-typhoid group, it presents many points of contrast, as regards its fermentative and pathogenic characters, to the species

*The results obtained with this anticoli serum are in marked contrast to those recorded by KRAUS in the *Wiener klinische Wochenschrift* (1901, 14, p. 693) which emphasize the specificity of the precipitating action of the serum of animals adapted to various races of the colon bacillus.

heretofore mentioned; namely, *B. typhosus*, *B. coli*, and the dextrose splitters of the intermediate group. The three races of *B. dysenteriae* employed for the production of antisera and filtrates were a *B. dysenteriae* (known as Flexner), the *B. dysenteriae*, Kruse, and the *B. dysenteriae*, New Haven (Duval). Mannite is not fermented by these races, which accordingly belong to the Shiga type of the *B. dysenteriae*. Unfortunately, only a few experiments could be made with those species or races of bacilli which have been mistaken for the *B. dysenteriae* Shiga; namely, those species which ferment mannite and monosaccharids, and those which, unlike the former race, ferment in addition maltose, dextrin, and saccharose. Both these races, unlike the *B. dysenteriae* Shiga, have only slight pathogenicity for rabbits.*

Difficulty was experienced in immunizing rabbits to the three races of the *B. dysenteriae* Shiga in our possession, living cultures being pathogenic, whereas boiled cultures in our hands conferred only slight protection, and the serum of rabbits adapted to boiled cultures yielded only traces of agglutinating and precipitating substances. A precipitating serum was, however, obtained finally in a rabbit with repeated doses of living cultures of *B. dysenteriae* "New Haven."

ANTIDYSENTERY RABBIT SERUM.

Rabbit received about ten agar cultures of *B. dysenteriae* "New Haven," between June 4 and August 12, 1902. Serum collected August 19.

Agglutinating value: 1-1,000.

1-10: distinct agglutination at $\frac{3}{4}$ hr.; complete at 4 hrs.

1-100: distinct agglutination at $\frac{3}{4}$ hr.; at $1\frac{1}{2}$ hrs., agglutination farther advanced than the dilution tube 1-10; complete at 4 hrs.

1-500: at $1\frac{1}{4}$ hrs., indistinct clumps; at 4 hrs., settling, heavy deposit at bottom; complete at 8 hrs.

1-1,000: slight settling at $1\frac{3}{4}$ hrs.; at 4 hrs., like 1-500; complete at 8 hrs.

No reaction in higher dilutions at 8 hours.

In the hanging drop with a dilution of 1-100, small clumps are formed at seven hours. With a dilution of 1-200, small clumps developed at one-half hour, which did not increase in size. The serum agglutinated the *B. dysenteriae* "Kruse" in a dilution of 1-500.

The filtrate of the *B. dysenteriae* "New Haven" was obtained from a broth culture of beef extract, grown for one hundred and two days at 37° C.

*The reader is referred to the interesting article of Hiss upon the differential fermentative peculiarities of the various species which have been thrown temporarily into the so called dysentery group;¹⁷ also LENTZ.¹⁸

TABLE XVI.

THE PRECIPITATING ACTION OF THE ANTIDYSENTERIC SERUM UPON ITS HOMOLOGOUS FILTRATES.

Filtrate "New Haven"	Antidysen. Serum "New Haven"	
1 c.c.	1 c.c.	Immediate clouding, and precipitation in a few minutes; complete at 4 hrs.
1	1/4	Immediate reaction.
1	1/10	Cloudiness developed after 15 min., at 37° C.; at 4 hrs., small flocculi on sides of tube.
5	1/10	No reaction at 10 hrs.; at 22 hrs., a flocculent precipitum.

The serum thus agglutinated in a 1-1,000 dilution and precipitated in a dilution of 1-50, which represents practically its maximal limit of precipitation.*

TABLE XVII.

THE PRECIPITATING ACTION OF THE ANTIDYSENTERIC SERUM UPON ITS HETEROLOGOUS FILTRATES.

1 c.c. colon filtrate + 1	c.c. dysenteric serum:	Immediate cloudiness; precipitum at 1 1/4 hrs.
1 " " " + 1/4 "	" " "	Immediate cloudiness; precipitum at 1 1/4 hrs.
1 " " " + 1/10 "	" " "	No instant reaction; faint cloudiness at 1 1/4 hrs.; precipitum at 4 hrs.
5 " " " + 1/10 "	" " "	Negative after 72 hrs.
1 " B.No.1 " + 1/4 "	" " "	Immediate cloudiness; at 4 hrs., abundant precipitum.
1 " " " + 1/10 "	" " "	Immediate cloudiness; at 4 hrs., cloudiness and slight precipitum.
5 " " " + 1/10 "	" " "	Negative at 72 hrs.

The typhoid filtrate reacted like the B. No. 1 filtrate. A prodigious filtrate was tested, with negative results.

SUMMARY.

The reactions above noted are not quantitatively as marked as that obtained with the homologous filtrate. The antidysenteric serum in a dilution of 1-50 failed to precipitate the filtrates of colon,

*Through the kindness of Professor Flexner, we were able to test the precipitating action of the serum of a horse which had undergone adaptation for four months with the dysentery bacillus—the culture "New Haven" isolated from a case of dysentery in an epidemic at New Haven.

Filtrate of *B. dysenteriae* "Flexner," 1/2 c.c. 1/10 and 1/4 c.c. horse serum.
 Filtrate of *B. dysenteriae* "Kruse," 1/2 c.c. 1/10 and 1/4 c.c. horse serum.
 Filtrate of *B. dysenteriae* "New Haven," 1/2 c.c. 1/10 and 1/4 c.c. horse serum.
 Tubes kept at 37° C.

No reaction at six hours, but after twenty-four hours a small amount of flocculent precipitate had developed. The serum added in the same proportions did not precipitate the filtrates of any of the colon-typhoid group in our possession. The low agglutinating value, 1-200, by microscopic test, of the horse serum, reasonably accounts for the failure to obtain precipitation in any other than the dysenteric filtrates, especially as it has been noted that the typhoid, colon, and our intermediate B. No. 1 antisera precipitated dysenteric filtrates.

typhoid, and B. No. 1. Thus a reaction was obtained with a serum dilution of 1-10, but not in 1-50, in these heterologous filtrates.

The B. dysenteriae "New Haven" having been shown to possess precipitative relationships for the B. typhosus, B. colon, and the intermediate B. No. 1, in the tests to be described the precipitative relationships between several species or races of the dysentery group were studied.

THE AGGLUTINATING AND PRECIPITATING ACTION OF THE SERA OF RABBITS ADAPTED TO THREE SPECIES OF THE DYSENTERY GROUP UPON THEIR CORRESPONDING AND UPON HETEROLOGOUS FILTRATES.

The three species of the dysentery group are classified (Hiss) according to their fermentation reactions in the following manner: (1) The B. Shiga ferments dextrose and other monosaccharids; but not mannit, maltose, dextrin, or saccharose. (2) The B. "Y" (Hiss) ferments monosaccharids and mannit, but not maltose, dextrin or saccharose. (3) The B. "Baltimore" (same type as Flexner's "Manila" and "Harris") ferments monosaccharids, mannit, maltose, dextrin, and saccharose.

Two dysentery filtrates were employed—a Kruse filtrate (90 days at 37° C.) and a B. "Y" (Hiss) filtrate (184 days at 37° C.). Through the kindness of Dr. Hiss, three antisera were placed at the writer's disposal—a "Shiga," a B. "Y" (Hiss), and a "Baltimore" antiserum. The "Shiga" antiserum and the "Kruse" filtrate may be considered homologous, since the B. Shiga and the B. Kruse are non-mannit factors and have similar agglutinating limits. For the Baltimore antiserum no homologous filtrate was at hand.

The following agglutination tests (Table XVIII) were made by Prof. Hiss with the three antisera of rabbits and also with an anti-typhoid serum* (Macroscopic method, readings at twenty-four hours at 37° C.).

SUMMARY.

Agglutination relationships were thus found to exist between the mannit splitters, the Baltimore and "Y" cultures. Since the normal agglutinins of rabbit serum occasionally reach 1-100 for each of these species, an agglutination reaction below 1-100 has no significance.

* These agglutination results were kindly placed at the writer's disposal for the sake of completeness of the experiments, although they are to appear later in an article by Prof. Hiss on agglutination in the "dysentery" group.

TABLE XVIII.

AGGLUTINATION TESTS.

(Tests made June 23, 1903.)

Shiga serum, bled June 17:	Shiga, 1-1,600-3,200. Baltimore, 1-400. "Y," 100. } Only a little above nor- Typhoid, 100. } mal rabbit serum.
Baltimore serum, bled May 20:	Shiga, 1-100. Baltimore, 1-3,200. "Y," \pm 400. Typhoid, \pm 100.
"Y" serum, bled April 30:	Shiga, -100. Baltimore, +1,600. "Y," 6,400. Typhoid, +100.
Typhoid serum, bled April and May:	Shiga, -100. Baltimore, +100-200. "Y," +100. Typhoid, +6,000.

PRECIPITATION TESTS.

The following precipitin reactions were made with the four antisera and the three filtrates:

TABLE XIX.

Fil. Kruse, 5.0 c.c.	0.1 c.c., "Y" serum.	Cloudy at 6 hrs.; same at 24 hrs.
" " 5.0 "	0.1 " Shiga "	Cloudy at 6 hrs.; same at 24 hrs.
" " 5.0 "	0.1 " typh. "	Negative at 24 hrs. and 48 hrs.
" " 5.0 "	0.1 " Balt. "	Negative at 24 hrs. and 48 hrs.
" " 0.9 "	0.1 " "Y" "	Cloudy at 1 hr.; small precipitum at 24 hrs.
" " 0.9 "	0.1 " Shiga "	Cloudy at 40 min.; small precipitum at 24 hrs.
" " 0.9 "	0.1 " typh. "	Negative at 24 hrs. and later.
" " 0.9 "	0.1 " Balt. "	Negative at 24 hrs. and later.
Fil. "Y," 5.0 c.c.	0.1 " "Y" "	Clouding in 40 min.; at 6 hrs., cloudy; slight precipitum at 24 hrs.
" " 5.0 "	0.1 " Shiga "	Clouding in 40 min.? at 6 hrs., cloudy; slight precipitum at 24 hrs.
" " 5.0 "	0.1 " typh. "	Negative at 24 hrs.
" " 5.0 "	0.1 " Balt. "	Negative at 24 hrs.
" " 0.9 "	0.1 " "Y" "	Almost immediate clouding, more at 40 min.; at 22 hrs., small precipitate.
" " 0.9 "	0.1 " Shiga "	Cloudiness at 40 min.; at 22 hrs., small precipitate.
" " 0.9 "	0.1 " typh. "	Negative at 24 hrs.
" " 0.9 "	0.1 " Balt. "	Negative at 24 hrs.
Fil. typh., Pfeiffer, 5.0 c.c.	0.1 " "Y" "	Negative at 24 hrs. and later.
" " " 5.0 "	0.1 " Shiga "	Negative at 24 hrs. and later.
" " " 5.0 "	0.1 " typh. "	At 6 hrs., cloudiness; at 24 hrs., small precipitate.
" " " 5.0 "	0.1 " Balt. "	Negative at 24 hrs.
" " " 0.9 "	0.1 " "Y" "	At 45 min., negative; at 6 hrs., negative; at 24 hrs., very small amount?
" " " 0.9 "	0.1 " Shiga "	At 45 min., negative; at 6 hrs., negative; at 24 hrs., small amount.
" " " 0.9 "	0.1 " typh. "	At 45 min., negative; at 6 hrs., small precipitate; at 24 hrs., small flocculent deposit.
" " " 0.9 "	0.1 " Balt. "	Negative at 45 min., at 6 hrs., and at 24 hrs.

The "Kruse" filtrate is precipitated by the homologous Shiga and "Y" serum, but not by the Baltimore or typhoid serum. (The typhoid serum was of low value.)

The "Y" filtrate is precipitated by "Y" and Shiga, but not by the Baltimore and typhoid antisera.

The typhoid filtrate is precipitated only by its corresponding serum in serum dilutions of 1-50; in 1-10 dilutions, also by Shiga and possibly by "Y" serum.

SUMMARY.

Marked precipitative relationships were thus shown to exist between the Shiga and "Y" bacilli; whereas no agglutinative relationships, or only very slight ones, could be demonstrated with the help of the corresponding antisera, which possessed, however, moderately low agglutinating values.

V. THE PRECIPITINS DEVELOPED IN RABBITS ADAPTED TO VARIOUS BACTERIAL SPECIES WHICH DO NOT BELONG TO THE COLON-TYPHOID GROUP.

This part of our theme was undertaken, first, to determine the absence or presence of adaptation precipitins for species of bacteria other than those of the colon-typhoid group in the sera of rabbits that had adapted themselves to these species; second, to determine the specificity of the precipitins thus formed for a species of a given group by testing the antisera upon the filtrates of species belonging to other groups as well as classes of bacteria. We thus endeavored to throw further light upon the extent of the biological relationships which exist among bacterial species of different groups and classes of bacteria, by means of the precipitin reaction, in a way similar to that which Nuttall¹⁹ has so successfully employed to demonstrate the biological relationships which exist between animals belonging to various orders, classes and groups.

We shall now consider the precipitins which were developed by adaptation to various species of bacteria not hitherto or only cursorily recorded in the literature, as well as the ineffectual attempts that were made to obtain precipitins for certain species of bacteria.

I. THE PRECIPITINS DEVELOPED BY ADAPTATION OF RABBITS TO B. PROTEUS.

The following tests were made with the serum obtained from a rabbit which had received six inoculations during a period of four weeks, at first with boiled and then with suspensions of living agar cultures. The agglutinating value of the serum was 1-5,000, complete in two hours; 1-10,000, incomplete at six hours. The precipitating action of the serum was slower and weaker than in the case of the antiprodigiosus and antistaphylococcus sera referred to below. One-fourth c.c. and 1/10 c.c. of the antiproteus serum added to 1 c.c. of the corresponding filtrate gave no reaction in five hours. Later a copious precipitum was formed in the tube containing the larger amount of serum and at twenty-four hours in the 1/10 c.c. tube the precipitum was still settling. One drop of serum to 1 c.c. gave no reaction at forty-eight hours at 37° C.

The antiproteus serum added to each of the filtrates in our possession likewise gave rise to no reactions, with the exception of the filtrate of the Sp. Metchnikovi, a slight reaction in this case being obtained at the end of twenty-four hours. On the other hand, the proteus filtrate was not precipitated or made cloudy by the antityphoid, coli, and B. No. 1 sera added in the proportion of 1/4 c.c. of serum to 1/2 c.c. of filtrate, or by antistaphylococcus or antipyocyaneus serum.

With another antiproteus serum, with an agglutinating value of 1-500, taken from a dying rabbit which had received two inoculations of proteus emulsion, a similar and confirmatory series of observations was made, the precipitating action of the serum, however, being slight.

We may therefore conclude that no precipitating relationships exist between the B. proteus and the bacilli of the colon-typhoid group and the pyogenic cocci, but that in the class of spirillaceae the Sp. Metchnikovi may give reactions.

II. THE PRECIPITINS DEVELOPED BY ADAPTATION OF RABBITS TO B. PRODIGIOSUS.

The antiprodigiosus sera yielded copious precipitates, and gave rise to the most rapid reactions which we have obtained with anti-bacterial sera.

The first serum was obtained from a rabbit inoculated at intervals, between April 4 and May 13, 1902, six, twenty-four-hour agar cultures being introduced into the peritoneum. The rabbit developed "snuffles"* May 16, and was therefore bled the next day. The serum had an agglutinating value of 1-10,000, complete in less than three hours. At 1-30,000 and 1-50,000 incomplete reactions were obtained.

THE PRECIPITATING ACTION OF ANTIPRODIGIOSUS RABBIT SERUM (No. 1)
UPON ITS HOMOLOGOUS FILTRATE.

One half c.c. and 1/10 c.c. of serum added to ½ c.c. of filtrate gave rise to instant cloudiness, heavy flocculi being formed in one-half hour, the reaction being complete in one hour.

THE PRECIPITATING ACTION OF VARIOUS ANTIBACTERIAL RABBIT SERA UPON
THE PRODIGIOSUS FILTRATE, AND THE ACTION OF ANTIPRODIGIOSUS RAB-
BIT SERUM (No. 1) UPON FILTRATES OF VARIOUS HETEROLOGOUS BAC-
TERIAL SPECIES.

The prodigious filtrate did not react with the various anti-bacterial sera in our possession; namely, anticolon, antityphoid, anti-B. No 1, and antidysentery; and no reaction followed the addition of the antiprodigious serum to filtrates of various bacilli, vibrios, and cocci; or this led to such small and inconstant precipitates, even when added in equal volumes, that these can be safely disregarded.

A second antiprodigious rabbit received eight twenty-four-hour agar cultures in the peritoneum, between April 7 and June 7, 1902, the rabbit being bled four days later. The macroscopic agglutination tests were instantaneous up to 1-500; 1-1,000, complete in less than one hour; 1-5,000, complete in less than two hours; 1-15,000, complete in six hours.

THE PRECIPITATING ACTION OF ANTIPRODIGIOSUS SERUM (No. 2) UPON ITS
HOMOLOGOUS FILTRATE.

One-half c.c. prodigious filtrate plus one drop of serum produced cloudiness and precipitation in two minutes, and at twenty-four hours a copious precipitum had formed. The limit of actual precipitation was obtained with a serum dilution of 1-300, the precipitum formed being very scanty.

* Adaptation with most of the cultures was rendered a tedious task during the winter months on account of a prolonged epidemic of "snuffles" in our rabbits. To forestall criticism, it may be mentioned here that only a few observations obtained with the sera of afflicted rabbits are recorded, the fact being noted in the text. Such sera were of value, however, in substantiating the results obtained with the sera of the healthy rabbits. The afflicted rabbits did not yield as potent precipitating and agglutinating sera, but the same relative results were found as with the antisera of healthy animals.

THE PRECIPITATING ACTION OF ANTIPRODIGIOSUS RABBIT SERUM (No. 2) UPON
THE FILTRATES OF VARIOUS ANTIBACTERIAL SPECIES.

The second antiprodigiosus serum was tested against various heterologous filtrates, each tube containing $\frac{1}{2}$ c.c. of the filtrate and $\frac{1}{4}$ c.c. of serum. The reactions obtained were slow, the twenty-four hour results alone being given. With filtrates of *B. typhosus*, *B. typhi murium*, *B. No. 1*, *B. psittacosis*, and *Sp. Metchnikovi*, a moderate amount of precipitum was formed, typical flocculi adhering to the sides of the tubes. The dysentery filtrates gave a small amount of precipitate. The filtrates of bovine and human tubercle bacilli, *B. icteroides*, and *Sp. cholerae*, were negative. At twenty-four hours all the fluids were clear and sterile, as found by cultural and microscopic tests of the fluid and precipitate.

The precipitin of this antiprodigiosus serum thus showing a somewhat marked affinity for the precipitable substances of these bacillary filtrates, one might be led to suppose that a strong precipitating serum would combine with the precipitable substance of any filtrate, whether of coccus or of bacillus, thus leading to the conclusion that there is no specificity in the precipitin reaction. This was readily disproved by the following test. The antistaphylococcus serum to be described later acted rapidly upon, and yielded a copious precipitum in, its corresponding filtrates.

TABLE XX.

Antiprodigiosus serum (No 2) 0.5 c.c.	Staphylococcus fil- trate 0.5 c.c.	After 48 hrs., at 37° C., absolutely negative.
Antistaphylococcus se- rum 0.5 c.c.	Prodigious filtrate 0.5 c.c.	After 48 hrs., at 37° C., absolutely negative.

Here, although both sera were extremely powerful, there is no interaction. In the course of the reactions between the antiprodigiosus serum and the above filtrates, but little of the precipitating substances of the serum were fixed, since addition of the prodigious filtrate to the tubes, when freed of the first precipitum that had formed after forty-eight hours contact at 37° C., gave rise to an immediate reaction. The reaction, although not delayed, was, however, never as copious. No attempt was made to calculate the amount of the precipitating substance of the serum which was thus fixed. This was the only antiprodigiosus serum obtained which precipitated heterologous filtrates of a different class, with the single exception of that of the filtrate of *Sp. Metchnikovi*.

A less actively precipitating serum of a rabbit treated with a sterile and cell-free prodigiosus filtrate likewise precipitated the *Sp. Metchnikovi* filtrate, a perfectly characteristic precipitate being formed in seventeen hours. The serum had no action on any other of the above filtrates, possibly because of its slighter precipitating action.

Our first antiprodigiosus serum obtained from a rabbit adapted by inoculation with living cultures, although of greater precipitating value for the corresponding filtrate than the serum of the rabbit inoculated with the prodigiosus filtrate, nevertheless failed to precipitate the same filtrate of *Sp. Metchnikovi*. From this fact it seems not unlikely that the *B. prodigiosus* develops several distinct precipitable substances, depending upon its environment. Thus, the filtrate of old broth cultures that was inoculated may have contained a larger quantity of the precipitable substance which gives rise to the formation of the special or partial precipitin of the *Sp. Metchnikovi*, than did the emulsion of the living bacilli with which the other rabbits had been inoculated. On the other hand, it is conceivable that there exists a difference, both quantitative and qualitative, in the cell receptors of different rabbits which give rise to the various partial precipitin haptines.*

Precipitative relationships between species of one group of Bacteriaceae and those of different groups of the same family and those of different families were thus found to exist. Since several equally actively precipitating antiprodigiosus sera did not precipitate the filtrates of the colon-typhoid group, it seems reasonable to assume that the cells of different rabbits develop precipitins which may vary qualitatively.

* Ascoli and von Dungern have noted qualitative differences in the hemo-precipitins developed in the sera of animals of the same and of different species. Von Dungern²⁰ has obtained almost conclusive evidence that the precipitable substance of the blood plasma of the *Major squinado* is composed of at least several haptophors which have distinct affinities, and that corresponding to the composite nature of this substance several precipitins are developed. The precipitins are thus composed of a number of partial substances, which are distinct from each other and react only with the special precipitable substance which has given rise to them. Joos²¹ has described two distinct agglutinins in anti-typhoid serum which, according to him, owe their origin to the presence in the typhoid bacillus of two distinct agglutinable substances. Joos's results seem capable of a different interpretation, however, from that which he has placed upon them.

III. THE PRECIPITINS DEVELOPED BY ADAPTATION OF RABBITS TO THE SPIRILLUM CHOLERAE AND METCHNIKOWI.

In the class of Spirillaceae only two species were investigated.

The first series of rabbits treated with cultures of *Sp. cholerae asiaticae* and *Sp. Metchnikovi* were unsuccessful, the sera having little precipitating action. The cholera culture was an old stock culture which had become non-pathogenic to guinea pigs and to rabbits. The *Sp. Metchnikovi* was, however, still pathogenic. Rabbits were finally adapted which yielded precipitating sera.

The anticholera rabbit was inoculated with twelve agar cultures (twenty-four hours' growth), in the course of ten weeks. (Agglutination test was impossible on account of extensive pseudo-clumping in broth cultures and precipitation of the emulsions of cholera cultures.) The anti-Metchnikovi rabbit received nine agar cultures grown for twenty-four hours at 37° C., in the course of seven weeks. The serum possessed an agglutinating value of 1-500, complete in four and one-half hours. The cholera serum did not agglutinate the *Sp. Metchnikovi* above the limit of normal rabbit serum (1-10 being nearly complete in four and one-half hours; 1-100 negative at twenty-four hours).

The filtrates of both species were obtained from broth cultures grown at 37° C. for fifty-one days.

TABLE XXI.

THE PRECIPITATING ACTION OF THE ANTICHOLERA AND ANTI-METCHNIKOWI SERA UPON CHOLERA AND METCHNIKOWI AND TYPHOID FILTRATES.

0.5 c.c. Cholera fil.*	0.5 c.c. cholera serum	No reaction up to 6 hrs.; at 24 hrs., a small precipitum in all tubes.
0.5 " " "	0.25 " " "	
0.5 " " "	0.1 " " "	
0.5 c.c. Metch. fil.	0.5 c.c. cholera serum	No reaction at 6 hrs.; at 24 hrs., a slight reaction, perhaps less than above homologous series.
0.5 " " "	0.25 " " "	
0.5 " " "	0.1 " " "	
0.5 c.c. Metch. fil.	0.5 c.c. Metch. serum	Instantaneous clouding in all tubes; at 4 hrs., reaction complete in all tubes; at 24 hrs., copious precipitum.
0.5 " " "	0.25 " " "	
0.5 " " "	0.1 " " "	
0.5 c.c. Cholera fil.†	0.5 cc. Metch. serum	Instantaneous clouding in all tubes. In 0.5 c.c. and 0.25 c.c. tubes copious precipitum, which did not form in 0.1 c.c. tube until 6 hrs. In the two sets of tubes at 24 hrs., same amount of precipitum.
0.5 " " "	0.25 " " "	
0.5 " " "	0.1 " " "	
0.5 c.c. Typhoid fil.	0.5 c.c. Metch. serum	No trace of reaction after 48 hrs., at 37° C.
0.5 " " "	0.5 c.c. cholera "	

* In the set of tubes which contained cholera filtrate and serum, where a slight reaction was first obtained, the addition of 0.5 c.c. of Metchnikovi serum likewise produced immediate cloudiness and precipitation in a few minutes. At eighteen to twenty-four hours there was a copious precipitum, showing that the cholera serum had fastened no appreciable amount of precipitable substance in its own filtrate.

† A further addition of 0.5 c.c. of Metchnikovi serum to the mixtures of cholera filtrate and Metchnikovi serum produced immediate cloudiness and precipitation at one and one-half hours; at eighteen to twenty-four hours, copious precipitum; most in 0.1 c.c. tube. The precipita were slightly greater than those of the homologous series.

DETERMINATION OF THE MAXIMAL PRECIPITATION LIMITS OF THE ANTI-METCHNIKOWI SERUM FOR ITS HOMOLOGOUS AND FOR THE CHOLERA FILTRATE.

The maximal limit of reaction of the anti-Metchnikowi serum on its corresponding filtrate was then determined.

TABLE XXII.

5 c.c. filtrate	1/10 c.c. serum.	Cloudiness within several hours; precipitation occurs before 20 hrs.
5 c.c. filtrate	1/20 c.c. serum.	Cloudiness is formed within 10 hrs.; never reaches a distinct precipitation even at 72 hrs.
5 c.c. filtrate	1/40 c.c. serum.	Negative at 72 hrs.

Maximal dilution about 1-100.

With 5 c.c. of cholera filtrate and 1/10 c.c. of Metchnikowi serum, no reaction at seventy-two hours. Maximal dilution at which precipitation still occurred in the cholera filtrate was considerably under 1-50, the exact limit not being determined.

SUMMARY.

From these tests the following conclusions can be drawn: The serum of an animal adapted to a given species of spirillum may precipitate the filtrate of another species of spirillum, although it does not agglutinate the spirillum. Distinct precipitative relationships may thus be present unassociated with agglutinative relationships.

IV. THE PRECIPITINS DEVELOPED BY ADAPTATION OF RABBITS TO *B. DIPHTHERIAE*.

It has long been known that during the process of immunization to certain species of bacteria the animal organism does not respond, or only with difficulty, with the production of precipitins, although various other antibodies, such as antitoxins, may be developed. We shall briefly describe our ineffectual attempts to obtain precipitins for the *B. diphtheriae*, the *B. pyocyaneus*, and the human and bovine tubercle bacillus.

No attempt was made to immunize rabbits with diphtheria toxin, as Kruse and Nicolle could not determine the presence of precipitins in diphtheria antitoxin (although agglutinins have been described in horse antitoxin serum). Although abundantly established that precipitable substances are present in filtrates, "toxins," the amount of precipitins developed in the serum of animals treated with filtrates was found by the writer and by other observers to be insignificant in comparison with that obtained when living bacterial cells are introduced.

Rabbits were accordingly immunized with emulsions of agar cultures, grown for twenty-four hours, in the hope of obtaining a precipitating or antibacterial serum. A rabbit was finally immunized against many times the fatal dose of diphtheria culture, preliminary doses of antitoxic horse serum being given. (The diphtheria bacillus was kindly furnished by the Department of Health of the city of New York, and is the one commonly designated throughout the world as the American bacillus, or Bacillus "8.")

Agglutination value of the serum: An emulsion was made from a thirty-hour agar culture in sterile saline solution. Macroscopic test:

1-10: reaction distinct in 40 min.

1-100, 1-500, and 1-1,000: complete in 4 hrs. at 37° C.

1-5,000: showed a greater settling than did the 1-10,000 or the control tube.

A second test failed to confirm the result above obtained, the emulsion being unsatisfactory on account of the clumping and sedimentation in the control tube. A third trial on June 14, ten days after the first test, was completely negative even in a 1-10 dilution. It seems reasonable to concede that the serum had a relatively low agglutinating value, about 1-1,000. The failures recorded in the second and third tests may perhaps be attributable to the condition of the emulsion.

THE PRECIPITATING ACTION OF THE ANTIDIPHTHERIA RABBIT SERUM UPON THE DIPHTHERIA FILTRATE.

The addition of 1/4 c.c. and 1/10 c.c. of serum to 1/2 c.c. tubes of diphtheria antitoxin (acid reaction), grown for three months at 37° C., caused no precipitation after several days at 37° C. Negative results were likewise obtained with a filtrate of extracts in normal salt solution of fresh agar cultures, even when mixtures of equal amounts (1 c.c.) of the antiserum and the solution were used.*

RÉSUMÉ OF PREVIOUS STUDIES UPON THE PRECIPITINS DEVELOPED BY ADAPTATION OF ANIMALS TO B. DIPHTHERIAE.

Diphtheria precipitins have been developed by S. Wassermann²² in the serum of rabbits treated with 0.1 per cent. ethylin-diamin extracts of dried and pulverized diphtheria bacilli, previously killed by heat, 60° C., any toxic action being neutralized by administration of suitable quantities of antitoxin.

Wassermann thus claims to have obtained a precipitating antidiphtheria serum which, unlike antitoxin, contains a substance which reacts specifically upon the body substance (the precipitable substance) of the diphtheria bacillus. The advantage claimed by him for this method over that by which merely the untreated bodies of the bacilli are inoculated is that more of the precipitable substance can be introduced. The serum, he claims, furnishes a method of differentiating the pseudo- from the true diphtheria bacilli by agglutination and precipitation tests. Wassermann hopes that with such a "bactericidal" serum the diphtheria bacillus may be made to disappear from the throats of patients who have continued to harbor the bacilli during a prolonged convalescence. Wassermann's expectation is premature, unless

* The diphtheria bacilli of three potato tube agar slants were grown for three days at 37° C. and emulsified in 5 c.c. of sterile normal saline solution and filtered.

one concedes that the precipitin is an immune body on a par in its action with the lysins, or is always accompanied by such a body, on which the assumption of the bactericidal powers of the serum is based.*

Lipstein²³ has shown that by a procedure similar to the one employed by us—intraperitoneal inoculation of living diphtheria bacilli mixed with anti-toxin to neutralize the toxin—a strong agglutinating serum is developed in rabbits. The subject of the agglutinin in antidiphtheria serum, and the employment of such sera to differentiate the true diphtheria races from the pseudo-diphtheria races or species, cannot be entered upon, the reader being referred to the articles of Lipstein and Lubowski,²⁴ and to the admirable article of Josef Schwöner.²⁵

Schwöner casually mentions that he has observed specific precipitation of broth diphtheria filtrates after twenty-four hours at 37° C., with anti-diphtheria horse serum.

V. THE PRECIPITINS DEVELOPED BY ADAPTATION OF RABBITS TO *B. PYOCYANEUS*.

With our stock culture of the *B. pyocyaneus* we were unable to obtain precipitating substances in the serum of several immunized rabbits for its homologous filtrates. The bacillus employed corresponded in most of its characters to the classical description of the *B. pyocyaneus*. With cultures of other races of this bacillus precipitins have been developed by Eisenberg.²⁷

VI. THE PRECIPITINS DEVELOPED BY ADAPTATION OF RABBITS TO HUMAN AND BOVINE TUBERCLE BACILLI.

Our efforts to obtain precipitating substances in the serum of rabbits which had been infected with living or inoculated with dead cultures of human and bovine tubercle bacilli were likewise unsuccessful, so that our experiments may be briefly given.

Rabbits inoculated with cultures of human and bovine tubercle bacilli, and bled at various periods of time after inoculation or before death, failed to develop precipitins in their sera for tubercle filtrates.

Five per cent. glycerin broth cultures grown for several months at 37° C., in which the growth of bovine and human bacilli had been luxuriant, were used as the test fluids. The rabbits were inoculated with virulent cultures or with large amounts of the surface scum from tubercle cultures. The sera tested were obtained from rabbits in various stages of tuberculization, from

*This is an interesting commentary on the loose methods of expression frequently employed in connection with the immune bodies, especially since A. Lipstein²⁶ was not able to obtain any evidence of the formation of a suitable amboceptor in the sera of animals inoculated with living cultures, although the same sera possessed marked agglutinating properties.

a local abscess at the site of inoculation in the subcutaneous tissue, to those with an acute general miliary tuberculosis of the peritoneum with more or less involvement of the lungs, lymph nodes, kidneys, and other organs. No suggestion of a precipitating reaction was obtained by addition of the serum to their corresponding filtrates. Negative results were also obtained with Koch's extract of tubercle bacilli, which he employed to determine the agglutinating action of the serum of animals and patients afflicted with tuberculosis. The solutions were made with the preparation of *zerriebene Tuberkelbacillus* prepared by the Farbwerk vom Meister Lucius und Bruning, Höchst a. M., the directions given for Koch's *Agglutinations-Flüssigkeit* being closely followed. Our sera, added in various proportions, did not precipitate or cause cloudiness of this solution.

VII. THE PRECIPITINS DEVELOPED BY ADAPTATION OF RABBITS TO THE STAPHYLOCOCCUS PYOGENES AUREUS, TO THE PNEUMOCOCCUS, AND TO THE STREPTOCOCCUS.

In the class of cocci three species were investigated as to their capacity for developing precipitins in the serum of rabbits which had been adapted by inoculation of their cultures. The species were a *Staphylococcus pyogenes aureus*, a pneumococcus, and a streptococcus. The staphylococcus precipitin is first described. The pneumococcus and the streptococcus are described together for brevity, since by their inoculation no precipitating sera were formed.

a) THE PRECIPITINS DEVELOPED BY ADAPTATION OF RABBITS TO THE STAPHYLOCOCCUS PYOGENES AUREUS.

The staphylococcus used during the course of the experiments was highly pathogenic for rabbits, and it was only with care that these were finally rendered immune.* With the serum obtained from a highly immunized rabbit, the following experiments were made:

The rabbit received from March 10 to April 7 nine agar cultures into the peritoneum. The agglutination tests with the serum were made by the macroscopic method with emulsions of eighteen to twenty-four-hour agar cultures.

- 1-100 and 1-500: complete in 5 hrs.
- 1-1,000: nearly complete in 5 hrs.
- 1-5,000: complete in 18 hours.
- 1-10,000: negative after 24 hrs.

1-5,000 may be said to be the limit of the agglutination value of the serum.

*I am indebted to Dr. Wadsworth for the culture, which was obtained from a case of severe phlegmonous inflammation of the nose.

TABLE XIII.

THE PRECIPITATING ACTION OF THE ANTISTAPHYLOCOCCUS SERUM UPON THE STAPHYLOCOCCUS FILTRATE.

Staphylococcus filtrate:	Antistaphylococcus serum	Immediate cloudiness, well marked after 5 min.; in 1 hr., distinct precipitate in sets of tubes at room as well as at incubator temperatures; reaction complete at 5 hrs., the filtrate being clear.
1/2 c.c.	1/4 c.c.	
1 c.c.	1/10 c.c.	

DETERMINATION OF THE MAXIMAL PRECIPITATION LIMITS OF THE ANTISTAPHYLOCOCCUS SERUM UPON THE STAPHYLOCOCCUS FILTRATE.

The maximal limit of dilution of the antistaphylococcus serum at which precipitation occurs in the staphylococcus filtrate was found to be a serum dilution of 1-60. The serum likewise precipitated the filtered normal saline extracts of fresh agar cultures, as shown below.*

THE PRECIPITATING ACTION OF ANTISTAPHYLOCOCCUS SERA UPON VARIOUS HETEROLOGOUS FILTRATES.

The above serum and other antistaphylococcus sera were tested upon the filtrates already referred to of the colon-typhoid group, and also upon the filtrates of *B. proteus*, *B. prodigiosus*, *Sp. cholerae*, and *Sp. Metchnikovi*, with negative results.

b) THE PRECIPITINS DEVELOPED BY ADAPTATION OF RABBITS TO THE PNEUMOCOCCUS AND TO THE STREPTOCOCCUS.

The precipitins for the pneumococcus and streptococcus are evidently, as shown by the failure to obtain any evidence of their formation in immunized rabbits, only obtained, if at all, by special procedures. Our experiments may therefore be briefly summarized. Three rabbits were rendered highly immune to virulent cultures of the pneumococcus and of the streptococcus† grown upon agar. The antisera of the rabbits failed to produce cloudiness or precipitates in their corresponding filtrates when tested in the following proportions: 0.5 c.c. filtrate + 0.25 c.c. serum. It might be

* Emulsions of the three-day growth on three agar tubes in 5 c.c. of sterile normal saline solution, after standing for several hours at room temperature, were passed through Berkefeld filters. Equal parts of the filtered solutions and antiserum, when mixed, gave rise to no reaction after several hours; at eighteen hours (37° C.) a copious precipitum was present.

† I am indebted to Prof. Hiss for the streptococcus which was obtained from a severe case of cellulitis of the foot. The pneumococcus was virulent to rabbits, and was obtained from Dr. A. Wadsworth, to whom my thanks are also due. The filtrates were obtained from 5 per cent. glycerin meat-infusion broth, grown for two months at 37° C.

concluded from these tests that the pneumococcus and streptococcus do not possess precipitable substances, or that, if present, suitable receptors are not present normally, or are sessile, or are not formed during the process of adaptation of rabbits to these species of cocci.

The following tests with *Staphylococcus pyogenes aureus*, indicate, however, that precipitable substances are present in the streptococcus and pneumococcus, but that these substances do not diffuse readily from their cells into their glycerin-broth cultures. This assumption furnishes an explanation of the non-precipitation of the filtrates by their corresponding antisera, although the same antipneumococcus and antistreptococcus sera precipitated the staphylococcus filtrate:

THE PRECIPITATING ACTION OF THE ANTIPNEUMO- AND ANTISTREPTOCOCCUS SERA UPON THE STAPHYLOCOCCUS FILTRATE.

0.5 c.c. staphylococcus filtrate + 0.25 c.c. serum.

The tests carried out in the above proportions show the formation of a small quantity of whitish, somewhat granular precipitate after eighteen to twenty-four hours. The tests were repeated several times with confirmatory results, the precipitum obtained being found free from bacteria on cultivation and on examination of the coverslips. In confirmation of the specific character of the precipitum obtained may be cited the fact that neither the antipneumococcus nor antistreptococcus sera produced precipitates in any of the filtrates of the colon-typhoid group or the other filtrates in our possession.

If the slight reactions obtained above are granted to be those of precipitation—and there seems to be no reasonable ground for doubting the correctness of this supposition—it seems fair to suppose that the precipitable substances exist in the pneumococcus and streptococcus. These substances, however, do not diffuse into the filtrates, being retained in the bodies of the cocci. When the living cultures are inoculated, they are set free and induce the formation of precipitins in the sera of the rabbits. These precipitins, however, possess only a relatively slight affinity for the precipitable substance in the staphylococcus, and hence the small reaction. The antipneumococcus and streptococcus sera do not therefore, produce precipitates in their corresponding filtrates, since little or no precipitable substances are present, these sub-

stances being retained in the bacterial cell protoplasm and not diffusing throughout the ambient fluid of the broth.*

Furthermore, it is reasonable to assume that no, or only an insufficient amount of, precipitable substance was present in the filtrate upon which the precipitins of our antipneumococcus and streptococcus sera could act with the formation of a precipitum, for the strongly precipitating antistaphylococcus serum does not yield precipitates in the pneumococcus and streptococcus filtrates.

The growth in the broth from which these filtrates were obtained was not as abundant as that formed in the staphylococcus or in other bacillary filtrates. The filtrates of staphylococcus, on the other hand, are rich in precipitable substances, and the precipitins of the pneumo- and streptococcus antisera possessing a slight affinity for the staphylococcus precipitable substance, precipitation follows.

RÉSUMÉ OF PREVIOUS STUDIES UPON THE PRECIPITINS DEVELOPED BY ADAPTATION OF ANIMALS TO THE PNEUMOCOCCUS AND TO THE STREPTOCOCCUS.

Pneumococcus.—Neufeld²⁸ obtained evidence of the formation of precipitating substances in the pneumococcus antisera by means of special procedures. According to him, the precipitable substance resides exclusively in the bacterial cells—a statement apparently true for the pneumococcus. By means of the solvent action of normal bile on a fresh pneumococcus culture, which does not alter the specific nature of the constituents of the cocci, a drop sufficing usually to dissolve the cocci of several cubic centimeters of broth culture, a solution is obtained to which, if a serum which agglutinates pneumococci in a dilution of 1-15 is added in the proportion of 1-2, 1-4, 1-8, refracting hyaline masses, the size of red corpuscles, are seen to form in the hanging drop after a quarter of an hour. The masses increase in size and clump, forming later a precipitate visible to the naked eye. Normal rabbit serum does not bring about this reaction. According to Neufeld, a serum which agglutinates specifically a bacterial species will always cause a precipitation in a solution containing a sufficient amount of the bacterial constituents. He leaves it uncertain whether one substance induces both reactions, as Kraus first claimed, or whether two distinct substances which are formed simultaneously in the serum induce these phenomena. Neufeld adheres to Bordet's theory of agglutination. Under the influence of the agglutinin a peculiar coagulation process takes place on the surfaces of the bacterial cells, which in the case of the pneumococcus is accompanied by visible changes in shape, and, secondly,

*A somewhat similar explanation has been given by Neufeld for the pneumococcus, by Aronson for the streptococcus, and by Schwöner and Wassermann for the precipitable substances of the diphtheria bacillus which is extracted from the cell protoplasm with difficulty. In our explanation the supposition is that the separation is made by the animal organism.

by an alteration in the physical properties of bacteria which induce clumping and change of equilibrium.

Dr. A. Wadsworth,²⁹ in our laboratory, has confirmed Neufeld's observation concerning the precipitation by antipneumococcus serum of the solution of the pneumococci which have been dissolved by bile.

Streptococcus.—Aronson³⁰ has shown that antistreptococcus sera which do not precipitate the ordinary streptococcus broth cultures nevertheless precipitate extracts of the cocci in 1 per cent. ethylin-diamin solutions. With a powerful antistreptococcus horse serum (for details the reader is referred to the original) he obtained typical agglutination of streptococcus cultures. The agglutination test, made by the macroscopic method, was absolutely typical and caused only by a serum of high valency. Complete reactions were obtained with dilutions of 1-30; those of 1-40 were slightly cloudy, and the 1-50 dilutions were negative. The clumps formed in the effective dilutions, after shaking, soon settled again, unlike the sedimentation occurring in cultures which remain cloudy. A 1-40 reaction was obtained with Aronson's so-called twentieth normal serum. Aronson believes that the agglutinating power of serum is independent of its protective power.

Besides the agglutinins, the serum contained a specific precipitin, but special methods for its demonstration were necessary, as the serum did not precipitate streptococcus broth filtrates. The bacterial cell-bodies were dried and crushed in a little water, or better, with ethylin-diamin solution, as described by Aronson in a previous article.³¹ The extract was filtered through paper until clear. Addition of his immune serum, in proportion of 1-10, to the solution thus prepared, caused precipitation in several hours; whereas normal serum caused no cloudiness. The method resembles that described by Robert Koch³² for the demonstration of the agglutinating and specific substances in the serum of individuals and animals. Aronson justly remarks upon the impropriety of Koch's designating this method one for the demonstration of agglutination instead of precipitation. How a supposedly sterile and cell-free solution can agglutinate is certainly incomprehensible. Aronson believes that his process of extraction will reveal precipitins in the various antibacterial sera which have hitherto not been successfully demonstrated by merely testing the sera upon broth filtrates.*

VI. THE PRECIPITATING ACTION OF THE ANTI-B. NO. 1, ANTI-TYPHOID, ANTICOLI, AND ANTIDYSENTERY SERA UPON THEIR HOMOLOGOUS AND HETEROLOGOUS SALINE SOLUTION EXTRACTS OF YOUNG AGAR CULTURES OF SPECIES OF THE COLON-TYPHOID GROUP.

Pick claims to have extracted from the typhoid bacillus two distinct substances, which he designated by the term "bacterio-coagulin." The bacterio-coagulin A was obtained from old broth filtrates, and is precipitated by alcohol. The bacterio-coagulin K was extracted from the normal saline extracts of young agar

*Tchistovitch reports that Marmorek has observed the precipitation of streptococcus filtrates by antistreptococcus serum.

cultures, and was not precipitated by alcohol from its aqueous solutions. Although Pick was not able to develop precipitins in the serum of rabbits which had undergone adaptation by the use of these substances when purified, his absorption tests led him to conclude that each of the two bacterio-coagulins nevertheless developed separate and distinct precipitins in the serum of animals adapted in the usual way to the typhoid bacillus. The action of these precipitins, sero-coagulins A and K, were specific, since they reacted only with their corresponding bacterio-coagulins. Granting that Pick's statements are correct, it would follow from our observations, referred to below, that precipitative affinities or relationships exist between the bacterio-coagulins A and K obtained from various species of the colon-typhoid group, the A body being found only in old broth filtrates, the K body only in the saline extracts of young agar cultures.*

Our observations may be briefly summarized. The sera previously referred to, which had been adapted to several species of the colon-typhoid group—namely, *B. typhosus*, *B. No. 1*, *B. coli*, and *B. dysentery*, "New Haven"—were employed. It was found that these antisera precipitated, not only the filtered normal saline solution extracts† of agar cultures, grown for three days at 37° C., of the homologous species, but also the extracts of heterologous species of this group. The reactions were slower in onset, and less copious precipitates were formed than in the case of old broth filtrates of these species.

VII. RELATIONSHIPS EXISTING BETWEEN THE BACTERIAL AGGLUTININS AND PRECIPITINS.

A résumé of the various arguments advanced concerning the identity or non-identity of the bacterial agglutinins and precipitins cannot be entered upon here. The following series of observations made with the antisera mentioned above and with other antisera may be briefly described, since they furnish further proof of the early views of French observers (Bordet, Tchistovitch) that

*Pick's statements regarding the specificity of bacterio-coagulins are in direct opposition to the results which the author has obtained in a series of experiments which will be referred to in a later article.

†The extracts were allowed to stand at room temperature or in cold storage, 5-8° C., for from 2-24 hours before filtration.

the bacterial agglutinins and precipitins are distinct substances. The antiserum of the B. No. 1, a dextrose fractor belonging to the intermediate "Durham" or hog cholera group, precipitated, it will be recalled, in high dilutions, not only the filtrates of the various species of this group, which has been subdivided into two types, corresponding to the two species of bacilli isolated by Schottmüller, and known as "Seemann" and "Müller" (Types A and B), but also in lower dilutions than the above, the following species, namely, the B. coli Escherich "non-saccharose fractor," B. typhi, and various species of the indefinite dysentery group.

The species of the intermediate group of the Seemann type, to which the B. No. 1 belongs—namely, B. "Seemann," B. psittacosis, B. Cushing, B. typhi murium, B. icteroides—possess marked agglutinative affinities, the species agglutinating with high dilutions of anti-B. No. 1 serum, the agglutinative and precipitative affinities exhibiting a striking parallelism. The species of the Müller type of the intermediate group—namely, B. Schottmüller, Müller, B. Gwyn, and B. enteritidis (type determination ?)—have slighter, and with some antisera practically no agglutinative affinities. The precipitative affinities of the species of this group, although less marked than those of the species of the "Seemann" type (1-10-40 instead of 1-80-150), nevertheless are relatively of a higher grade and are more general than are their corresponding agglutinative affinities, as mentioned above.

Our contention as to the distinctness of the agglutinins and precipitins is confirmed by our observations that several anti-B. No. 1 sera did not agglutinate B. typhi or the B. coli (Escherich type), although these sera precipitated copiously the typhoid and colon filtrates in dilutions above 1-10, but not in dilutions of 1-50.

In this connection the agglutinative and precipitative relationships of antisera developed for the two types of bacilli isolated by Schottmüller, for several species of the intermediate and dysentery groups are of interest. The anti-"Seemann" serum agglutinated B. "Seemann" 1-100,000; B. paracolon [Libmann] and B. No. 1, 1-64,000; B. typhi murium, B. psittacosis, and B. icteroides, 1-32,000; B. Cushing and a hog cholera bacillus designated by Professor Smith* as "motile," 1-16,000; and the following species which belong possibly to the Müller type of the intermediate group: B. "Müller,"

* We are indebted to Professor Theobald Smith for his motile and non-motile species of hog cholera, both cultures being dextrose splitters belonging to the intermediate group.

1-50 (large motile clumps and scanty sedimentation, negative 1-200, microscopic method); B. Gwyn, 1-50, negative 1-200; and B. enteriditis, 1-200; also the following species: B. suipestifer (Král's) (hog cholera-Salmon-Smith), 1-16,000; B. suisepcticus (Král's), 1-1,000 (?); and the non-motile species of hog cholera of Professor Smith, negative 1-50; B. dysenteriae "Y" Hiss, 1-250; B. dysenteriae Kruse, negative 1-50; B. coli (Escherich), 1-100; B. typhi, 1-100.*

Owing to unavoidable circumstances we were able to test the precipitating action of the anti-Seemann serum upon only two filtrates of the species mentioned above. The maximal limit of precipitation for the homologous B. "Seemann" filtrate was 1-100, for the B. "Müller" filtrate, 1-40.† We see again illustrated the independence of the agglutinating and precipitating activities of antisera, the "Seemann" serum precipitating the "Müller" filtrate in dilutions of 1-40, whereas it fails to agglutinate the B. "Müller" in dilutions of 1-50, the homologous bacillus "Seemann" being agglutinated in dilutions of 1-10,000.

With the anti-"Müller" serum the following observations were made: The serum agglutinated B. "Müller" 1-16-32,000, and B. Gwyn 1-8-16,000, but did not agglutinate in dilutions of 1-50, B. "Seemann," B. No. 1, B. psittacosis, B. icteroides, B. typhi murium, B. paracolon Libman, and B. Cushing. The motile species of Professor Smith were positive 1-50, negative 1-500; the non-motile species, negative 1-50; likewise B. enteriditis; B. coli "Escherich," positive 1-500; B. typhi murium, positive 1-500; B. dysenteriae, "Y" Hiss and B. dysenteriae, "Diamond" Hiss, both negative 1-50; B. dysenteriae, Kruse, positive 1-50.

On the other hand, the antiserum developed cloudiness and flocculi within ten hours in dilutions of 1-10 in the following filtrates of the species above mentioned, which it did not agglutinate in dilutions of 1-50: B. Schottmüller "Seemann," B. Cushing B. No. 1, B. psittacosis, and B. dysenteriae "Y" Hiss, as well as in its homologous filtrates.‡

We thus see that, although B. No. 1 and B. Schottmüller "Seeman" cannot

* The tests were made in tubes of narrow caliber with agar cultures grown for 24 hours at 37° C., suspended in normal salt solution, 10 c.c. for one agar culture, readings being made at 2, 10 and 19 hours. Those recorded represent the extreme limits—microscopic clumps seen with low-power lens at 19 hours. Equal parts ½ c.c. of emulsion and serum dilution were employed.

† The filtrates were obtained from broth cultures of similar periods of incubation, 79 days at 37° C.

‡ Although the writer is of the opinion that his contention as to the distinctness of the bacterial agglutinins and precipitins, and consequently of the corresponding substances (agglutinable and precipitable substances), has been placed on a sound basis, nevertheless he realizes that many points underlying the intimate processes of agglutination and precipitation are still unknown. To illustrate, the following agglutination limits of an anti-B. No. 1 serum kept in cold storage for two years and greatly concentrated through evaporation, may be cited:

B. No. 1, 1-40,000, practically complete and clear at eighteen hours at 37° C.; microscopic clumps seen with aid of hand lens at 1-60,000. (No proagglutinoid zone at 1-20.)
 B. psittacosis: complete, 1-20,000; microscopic, 1-80,000.
 B. Schottmüller "Seemann": complete, 1-40,000; microscopic, 1-80,000.
 B. Schottmüller "Müller": complete, 1-4,000; microscopic, 1-80,000.
 B. Cushing: complete, 1-8,000; microscopic, 1-80,000.
 B. Gwyn: complete, 1-2,000; microscopic, 1-80,000.

be differentiated by their agglutinative reactions or biochemical characters, they nevertheless develop antisera with marked agglutinating differences indicating differences in the precipitable substances of the two species of bacilli, which may be explained, possibly, by the multiplicity of their precipitable substances, or of their haptophor groups.

The following observations made upon the agglutinating properties of the anti-B. No. 1 serum for the typhoid bacillus are of interest.

An anti-B. No. 1 serum, with an agglutinating value of 1-10,000, agglutinated the typhoid bacillus in dilutions up to 1-2,000; another anti-B. No. 1 serum agglutinated the same typhoid bacillus incompletely in dilutions of 1-300; whereas a third antiserum failed to agglutinate in dilutions above 1-10. On the other hand, three different antityphoid sera possessed no agglutinating properties for the B. No. 1, although the three antisera possessed marked precipitating action upon the filtrates of the B. No. 1.*

Observations similar to those recorded above concerning the distinctness of the two substances as seen by the non-parallelism of their activities, expressed in terms of serum dilutions, were made in the case of the antityphoid sera which in dilutions of 1-10, and in greater dilutions in the case of some filtrates, precipitated all the filtrates of the species belonging to the colon-typhoid group, enumerated in the text, whereas the serum (1-20,000) agglutinated the B. enteritidis (limit, 1-300), the other species being clumped in much lower dilutions or in such as approach approximately the limits of normal rabbit sera.

To conclude: an anticolon serum (agglutinating value, 1-250) precipitated the colon-filtrate in a dilution of 1-500; and in a dilution of 1-50 all the filtrates of the colon-typhoid group mentioned above, except B. Gwyn and B. Schottmüller (Seemann).

*Many observers have called attention to numerous examples of the specificity of the agglutinins of various species of the intermediate group. Durham, Castellani, and other observers have, on the other hand, noted numerous examples of agglutinative relationships between the members of the colon-typhoid group. See BELJAEFF, "Ueber Paratyphus-Erkrankungen."³³

The reader is referred to a most suggestive article upon the agglutination affinities of related bacteria by Theobald Smith and Reagh.³³ According to them, "there exist agglutination relationships between the pathogenic groups of bacilli which ferment dextrose. . . . This relationship is not brought out clearly unless the agglutinative limit of the various cultures is worked out with a serum agglutinating its specific bacillus in dilutions of one to one thousand and above."

On the other hand, the serum agglutinated only in low dilutions the homologous colon cultures, and was without action upon another culture of *B. coli*, as well as upon the intermediate *B. No. 1* and the *B. typhosus*.

A consideration of these observations leads us to the conclusion that the bacterial agglutinins and precipitins are distinct substances which owe their origin in the serum of adapted animals to different bacterial receptors or haptophor groups. According to Ehrlich's theory, the haptophor groups are alone responsible for the development of the immune bodies; hence the assumption that the substances in question are identical would leave wholly unexplained the fact that an antiserum may possess precipitative affinities of a high degree without or with only slight agglutinative affinities for a given heterologous species, or even for its homologous species, as in the case of one of the anticoli sera, or only in low dilutions with the other sera. On the other hand, if it be assumed that the substances owe their origin to different sets of receptors, we have only to assume that the haptophors (precipitable substances) which give rise to the partial precipitins* are more constantly present in the bacterial cell than are the corresponding haptophors (partial agglutinable substances) which give rise to the partial agglutinins.

VIII. CONCLUSIONS.

The following conclusions may be drawn from our researches:

1. The normal serum of the rabbit and of the ox, and various antibacterial rabbit sera, exert no precipitating action upon the usual peptone-salt meat infusions "or Liebig's extract," broth of slight alkaline or acid reaction.

Normal rabbit serum and ox serum do not precipitate bacterial broth filtrates. We are therefore warranted in drawing the conclusion that bacterial precipitins are absent in the serum of the rabbit and of the ox.

- 2 a) The sera of rabbits adapted respectively to four species of the colon-typhoid group, which species are distinguished from

* Ehrlich and Morgenroth have assumed the existence of partial hemolysins; Durham and Wassermann, of partial bacterial agglutinins; and von Dungern, of partial hemoprecipitins.

each other by definite and constant biochemical characters—namely, *B. typhosus*, *B. No. 1*, a member of the hog-cholera or intermediate group of Durham, *B. coli communis*, “Escherich,” and *B. dysenteriae* Shiga (New Haven “Duval”)—precipitate not only their homologous filtrates, but also the filtrates of the species above mentioned, as well as the filtrates of all the species of the colon-typhoid group which were tested by us; namely, the filtrates of *B. psittacosis*, *B. typhi murium*, *B. enteritidis*, *B. icteroides*, *B. paracolon* Gwyn, *B. paracolon* Cushing, *B. Schottmüller*, “Müller,” and “Seemann.”

The reactions obtained by the sera of rabbits adapted to one species of the colon-typhoid group in its homologous filtrate and in the heterologous filtrates vary in the time of onset of the reaction, as denoted by the appearance of cloudiness, and in the copiousness of the precipitum which finally develops, cloudiness developing more quickly, and the amount of precipitum formed being greater, in the homologous than in the heterologous filtrates. These differences are most strikingly exhibited when actively precipitating antisera are tested in higher dilutions; for an antiserum invariably precipitates its homologous filtrate in higher dilutions than the filtrates of heterologous species—a fact which may be made use of in the differentiation of bacterial species.

The precipitins for species of this group, cannot, however, be considered specific in the strict sense, but rather generic; for, as mentioned above, a serum adapted to one species precipitates in low serum dilutions the filtrates of heterologous species. The bacterial precipitins may thus serve to indicate genus or group relationship. The term “specificity” has merely a quantitative and not a qualitative value, and should be applied only in this broad manner, for the bacterial precipitins.

The quantitative differences in the precipitating action of the sera of rabbits which have been adapted to various species of the colon-typhoid group for the filtrates of homologous and heterologous species of this group are best explained, we believe, at least for the present, by the assumption that mutual bacterial receptors—precipitable substances—exist among the species of

one group of bacteria, which receptors give rise to the formation of partial precipitins in the sera of adapted rabbits.

b) The sera of rabbits adapted respectively to two species of the group of Spirillaceae—namely, *Sp. cholerae asiae*, and *Sp. Metchnikovi*—precipitate not only the homologous filtrate, but also the filtrates of the other species. The statements made above concerning the precipitins of the colon-typhoid group would seem to apply to the precipitins developed by the use of species of the genus of Spirillaceae.

c) In regard to the Coccaceae, we have reason to believe that precipitative relationships also exist between certain groups of this family, for slight precipitates were developed in the filtrates of *Staphylococcus pyogenes aureus* by antipneumococcus and antistreptococcus sera.

3. Precipitative relationships between species belonging to one group of Bacteriaceae for those of different groups of the same family, or for those of different families, such as the Coccaceae and Spirillaceae, as a rule, were found not to exist. Exceptions to this general rule were, however, noted. Thus, the serum of a rabbit adapted to a species of one group (*B. prodigiosus*) may precipitate the filtrates of many different species belonging to other groups (colon-typhoid), and also those of species (*Sp. Metchnikovi*) of a different family (Spirillaceae). The precipitative relationships are, however, limited. Thus the same antiprodigiosus serum had no precipitative action upon filtrates of species (*B. proteus*) of other groups of the same family (Bacteriaceae), or of a different family (*Staphylococcus pyogenes aureus*).

These facts lead us to believe that the precipitin reaction may serve to detect biological relationships between various groups and families of bacteria analogous to those which Nuttall and others have shown to exist in the animal kingdom.

4. From a consideration of the agglutinative relationships among members of the colon-typhoid group described by various observers, and from our limited number of observations and of the precipitative relationships described in our paper, the following conclusion may be safely drawn: The precipitative relationship between various species of one group of bacteria is a much

more intimate and constant one than the agglutinative relationship. The correctness of this assumption is supported and emphasized by the fact that the reaction of precipitation, as applied to bacterial filtrates, occurs only with low serum dilutions—as a maximum 1–500, but usually much lower; whereas agglutination readily occurs with thousandth dilutions. Furthermore, although agglutination and precipitation are usually closely associated phenomena, they are independent. In other words, the agglutinins and the precipitins are distinct substances.

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